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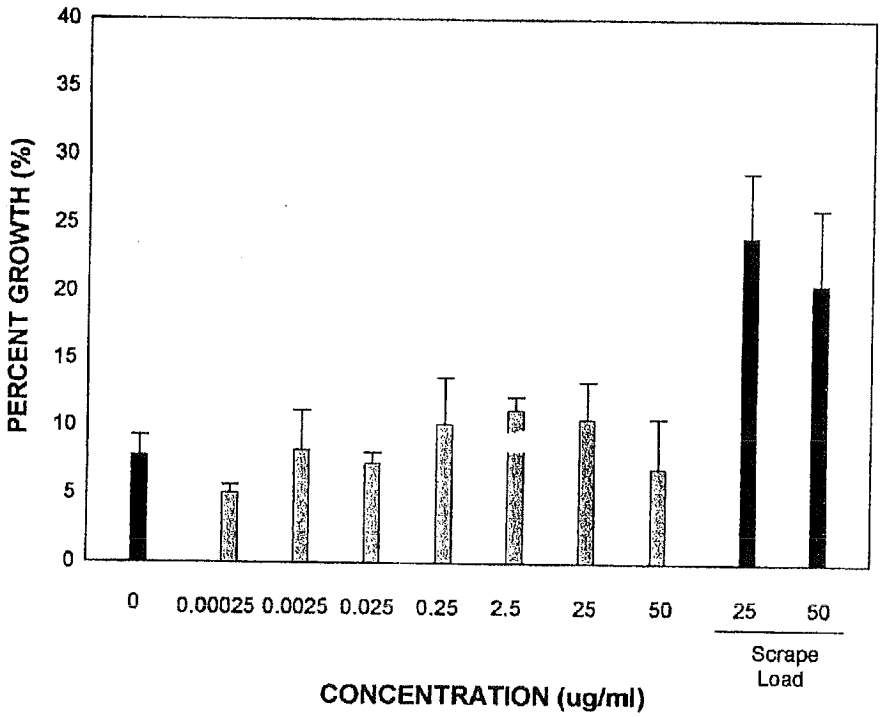
(71) Demandeur/Applicant:
MCKERRACHER, LISA, CA

(72) Inventeur/Inventor:
MCKERRACHER, LISA, CA

(74) Agent: BROUILLETTE KOSIE

(54) Titre : PROTEINES DE FUSION
(54) Title: FUSION PROTEINS



(57) Abrégé/Abstract:
The Rho family GTPases regulate axon growth and regeneration. Inactivation of Rho with C3, a toxin from Closteridium botulinum, can stimulate regeneration and sprouting of injured axons. The present invention provides novel chimeric C3-like Rho antagonists. These new antagonists are a significant improvement over C3 compounds because they are 3-4 orders of magnitude more potent to stimulate axon growth on inhibitory substrates than recombinant C3. The invention further provides evidence that these compounds promote repair when applied to the injured mammalian CNS.

TITLE: FUSION PROTEINS

ABSTRACT

The Rho family GTPases regulate axon growth and regeneration. Inactivation of Rho with C3, a toxin from *Closteridium botulinum*, can stimulate regeneration and sprouting of injured axons. The present invention provides novel chimeric C3-like Rho antagonists. These new antagonists are a significant improvement over C3 compounds because they are 3-4 orders of magnitude more potent to stimulate axon growth on inhibitory substrates than recombinant C3. The invention further provides evidence that these compounds promote repair when applied to the injured mammalian CNS.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: LISA MCKERRACHER

(ii) TITLE OF INVENTION: FUSION PROTEINS

(iii) NUMBER OF SEQUENCES: 35

(iv) CORRESPONDENCE ADDRESS:
(A) ADRESSEE: BROULLETTE KOSIE
(B) STREET: 1100 RENE-LESVEQUE BLVD WEST
(C) PROV/STATE: QUEBEC
(D) COUNTRY: CANADA
(E) POSTAL/ZIP CODE: H3B 5C9

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE:ASCII (TEXT)

(vi) CURRENT APPLICATION DATA:
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(vii) ATTORNEY/AGENT INFORMATION:
(A) NAME: BROULLETTE KOSIE
(B) REGISTRATION NO.:
(C) REFERENCE/DOCKET NO.: 06447-004-CA-01
(D) TEL. NO.: (514) 397 8500
(E) FAX NO.: (514) 397 8515

(2) INFORMATION FOR SEQ ID NO: 1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 BASE PAIRS
(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE: 5' cDNA primer - C3 sequence

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
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(D) OTHER INFORMATION:

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(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

5'GAA TTC TTT AGG ATT GAT AGC TGT GCC 3'

(2) INFORMATION FOR SEQ ID NO: 2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 BASE PAIRS

(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE: 3' cDNA primer - C3 sequence

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
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(x) PUBLICATION INFORMATION:
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(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

5'GGT GGC GAC CAT CCT CCA AAA 3'

(2) INFORMATION FOR SEQ ID NO: 3:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 888 BASE PAIRS
(B) TYPE: cDNA
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY: C3APL
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
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(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3: (Nucleotide sequence of protein C3APL)

GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA
AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA
GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA
AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA
AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA
AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA

AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT
CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA
AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT AGA CTT GAA TAT GGA TAT ATT
AGT ACT TCA TTA ATG AAT GTC TCT CAA TTT GCA GGA AGA CCA ATT ATT ACA
CAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT AGT
GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT
ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA
ACA ATG ATG GGC ACA GCT ATC AAT CCT AAA GAA TTC GTG ATG GAA TCC CGC
AAA CGC GCA AGG CAG ACA TAC ACC CGG TAC CAG ACT CTA GAG CTA GAG
AAG GAG TTT CAC TTC AAT CGC TAC TTG ACC CGT CGG CGA AGG ATC GAG ATC
GCC CAC GCC CTG TGC CTC ACG GAG CGC CAG ATA AAG ATT TGG TTC CAG AAT
CGG CGC ATG AAG TGG AAG AAG GAG AAC TGA

(2) INFORMATION FOR SEQ ID NO: 4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 295 AMINO ACIDS
(B) TYPE: PROTEIN
(C) STRANDEDNESS SINGLE:
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
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(B) TITLE:
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(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4: (amino acid sequence of C3APL)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSE
KEAIVSYTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPE
NIMLFRGDDPAYLGTEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGY
ISTSLMNVSQFAGRPIITQFKVAKGSKAGYIDPISAFQGQLEMLLPRHST
YHIDDMRLSSDGKQIIITATMMGTAINPKEFVMESRKRARQTYTRYQTLE
LEKEFHFNRYLTRRRRIEIAHALCLTERQIKIWFQNRRMKWKKEN

(2) INFORMATION FOR SEQ ID NO: 5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 774 BASE PAIRS
(B) TYPE: cDNA
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
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(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5: (Nucleotide sequence of C3APS)

GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT AAA GAA TTC CGC CAG ATC AAG ATT TGG TTC CAG AAT CGT CGC ATG AAG TGG AAG AAG GTC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA

(2) INFORMATION FOR SEQ ID NO: 6:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 257 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
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(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:(amino acid sequence of C3APS)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSE
KEAIVSYTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPE
NIMLFRGDDPAYLGTEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGY
ISTSLMNVSQFAGRPIITQFKVAKGSKAGYIDPISAFQGQLEMLLPRHST
YHIDDMRLSSDGKQIIITATMMGTAINPKEFRQIKIWFQNRRMKWKKVDS
SGRIVTD

(2) INFORMATION FOR SEQ ID NO: 7:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 BASE PAIRS
(B) TYPE:
(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: 5' cDNA primer - antennapedia sequence

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
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(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7: (EX 3)

5'GAA TCC CGC AAA CGC GCA AGG CAG 3'

(2) INFORMATION FOR SEQ ID NO: 8:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 BASE PAIRS
(B) TYPE:NUCLEIC ACID
(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: 3' cDNA primer

(v) FRAGMENT TYPE: antennapedia sequence

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
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(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8: (EX 3)

5'TCA GTT CTC CTT CTT CCA CTT CAT GCG 3'

(2) INFORMATION FOR SEQ ID NO: 9:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 BASR PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY:LINEAR

(ii) MOLECULE TYPE: OLIGONEUCLEOTIDE STRAND 1

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
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(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9: (EX 3)

5'AAT TCC GCC AGA TCA AGA TTT GGT TCC AGA ATC GTC GCA TGA AGT GGA AGA AGG 3'

(2) INFORMATION FOR SEQ ID NO: 10:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 54 BSE PAIRS
(B) TYPE:NUCLEIC ACID

(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: OLIGONUCLEOTIDE STRAND 2

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
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(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10: (EX 3)

5'GGC GGT CTA GTT CTA AAC CAA GCT CTT AGC AGC GTA GTT CAC CTT CTT CCA GCT 3'

(2) INFORMATION FOR SEQ ID NO: 11:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: TAT 5' PRIMER

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
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(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

5'GAATCCAAGCATCCAGGAAGTCAGCC 3'

(2) INFORMATION FOR SEQ ID NO: 12:
(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:TAT 3' PRIMER

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
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(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12: (ex 8)

5' ACC AGC CAC CAC CTT CTG ATA 3'

(2) INFORMATION FOR SEQ ID NO: 13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 876 BASE PAIRS

(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3-TL SEQUENCE

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
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(C) JOURNAL:
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(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13: (DNA sequence of C3-TL)

GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA
AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA
GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA
AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA
AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA
AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA
AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT

CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT AAA GAA TTC AAG CAT CCA GGA AGT CAG CCT AAA ACT GCT TGT ACC AAT TGC TAT TGT AAA AAG TGT TGC TTT CAT TGC CAA GTT TGT TTC ATA ACA AAA GCC TTA GGC ATC TCC TAT GGC AGG AAG CGG AGA CAG CGA CGA AGA GCT CAT CAG AAC AGT CAG ACT CAT CAA GCT TCT CTA TCA AAG CAG TAA

(2) INFORMATION FOR SEQ ID NO: 14:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 291 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3-TL PROTEIN SEQUENCE

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
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(B) TITLE:
(C) JOURNAL:
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(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14: (The protein sequence of C3-TL)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVSYT
KSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPENIMLFRGDDPAYLGTEFQ
NTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITQFKVAKGSKA
GYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFKHPGSQPK
TACTNCYCKKCCFHCQVCFITKALGISYGRKRRQRRRAHQNSQTHQASLSKQ

(2) INFORMATION FOR SEQ ID NO: 15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: TS OLIGONUCLEOTIDE STRAND1

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:

(B) TITLE:
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(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15: (EX 9)

5'AAT TCT ATG GTC GTA AAA AAC GTC GTC AAC GTC GTC GTG 3'

(2) INFORMATION FOR SEQ ID NO: 16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: TS OLIGONUCLEOTIDE STRAND 2

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:

(B) TITLE:
(C) JOURNAL:
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(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16: (EX 9)

5' GAT ACC AGC ATT TTT TGC AGC AGT TGC AGC AGC ACA GCT 3'

(2) INFORMATION FOR SEQ ID NO: 17:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 756 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3-TS cDNA

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:

(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17: (Nucleotide sequence of C3-TS)

GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT AAA GAA TTC TAT GGT GCT AAA AAA CGT CGT CAA CGT CGT CGT GTC GAC TCG AGC GGC CCG CAT CGT GAC TGA

(2) INFORMATION FOR SEQ ID NO: 18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 251 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3-TS PROTEIN

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18: (The protein sequence of C3-TS)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVSYT
KSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPENIMLFRGDDPAYLGTEFQ
NTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITQFKVAKGSKA
GYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFYGAKKRRQ
RRRVDSSGPHRD

(2) INFORMATION FOR SEQ ID NO: 19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:
(B) TYPE:
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19: (EX 10)

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGACTT
CTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGT
GATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCTTAT
TATATTGATGGTGATGTTAAATTAACACAGTCTATGGCCATCATACGTTATATAGCT
GACAAGCACAACATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTT
GAAGGAGCGGTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGAC
TTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTC
GAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCATCCTGAC
TTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCCAATGTGCCTGGATG
CGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTGAAGCTATCCCACAAATTGATA
AGTACTTGAAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGT

TTGGTGGTGGCGACCATCCTCCAAAATCGGATCTGGTTCCGCGTGGATCCTCTAGAG
TCGACCTGCAGGCATGCAATGCTTATTCCATTAATCAAAAGGCTTATTCAAATACTT
ACCAGGAGTTTACTAATATTGATCAAGCAAAAGCTTGGGGTAATGCTCAGTATAAAA
AGTATGGACTAAGCAAATCAGAAAAAGAAGCTATAGTATCATATACTAAAAGCGCT
AGTGAAATAAATGGAAAGCTAAGACAAAATAAGGGAGTTATCAATGGATTTCCTTC
AAATTTAATAAAACAAGTTGAACTTTTAGATAAATCTTTTAATAAAATGAAGACCCC
TGAAAATATTATGTTATTTANAGGCGACGACCCTGCTTATTTAGGAACAGAATTTCA
AAACACTCTTCTTAATTCAAATGGTACAATTAATAAAACGGCTTTTGAAAAGGCTAA
AGCTAAGTTTTTAAATANAGATAGACTTGAATATGGATATATTAGTACTTCATTAAT
GAATGTTTCTCAATTTGCAGGAAGACCAATTATTACAAAATTTAAAGTAGCAAAAGG
CTCAAAGGCAGGATATATTGACCCTATTAGTGCTTTTCAGGGACAACTTGAAATGTT
GCTTCCTAGACATAGTACTTATCATATAGACGATATGAGATTGTCTTCTGATGGTAA
ACAAATAATAATTACAGCAACAATGATGGGCACAGCTATCAATCCTAAAGAATTCA
GAAGGAAACAAAGAAGAAAAAGAAGACTGCAGGCGGCCGCATCGTGA

(2) INFORMATION FOR SEQ ID NO: 20: (EX 10)
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:
(B) TYPE:
(C) STRANDEDNESS:
(D) TOPOLOGY:

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20: (EX 10)

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYID
GDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKV
DFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALD
VVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKS
DLVPRGSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEA
IVSYTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKM
KTPENIMLFXGDDPAYLGTEFQNTLLNSNGTINKTAFEKAKAKFLNXDRLEYGYISTSLM
NVSQFAGRPIITKFKVAKGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIIT
ATMMGTAINPKEFRRKQRRKRRLQAAAS

(2) INFORMATION FOR SEQ ID NO: 21:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:16 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC1 TRANSPORT SEQUENCE

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21: (C3basic1)

K R R R R R P K K R R R A K R R

(2) INFORMATION FOR SEQ ID NO: 22:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:48 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: OLIGONUCLEOTIDE STRAND 1

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22: (EX 12)

AAG AGA AGG CGA AGA AGA CCT AAG AAG AGA CGA AGG GCG AAG AGG AGA

(2) INFORMATION FOR SEQ ID NO: 23:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY:LINEAR

(ii) MOLECULE TYPE: C3BASIC1 OLIGONUCLEOTIDE STRAND 2

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23: (EX 12)

TTC TCT TCC GCT TCT TCT GGA TTC TTC TCT GCT TCC CGC TTC TCC TCT

(2) INFORMATION FOR SEQ ID NO: 24:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 792 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC1

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24: (EX 12 DNA sequence)

GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA AAT ATT ATG TTA TTT ANA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT ANA GAT AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT AAA GAA TTC AAG AGA AGG CGA AGA AGA CCT AAG AAG AGA CGA AGG GCG AAG AGG AGA CAC CAC CAC CAC CAC CAC GTC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA

(2) INFORMATION FOR SEQ ID NO: 25:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 263 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC1 PROTEIN SEQUENCE

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25: (EX 12 Protein sequence)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVSYT

KSASEINGKLRQNKGVINGFPS
NLIKQVELLDKSFNKMKTPENIMLFXGDDPAYLGTEFQNTLLNSNGTINKTAFEKAKAKF
LNXDRLEYGYISTSLMNVSQ
FAGRPIITKFKVAKGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATM
MGTAINPKEFKRRRRRPKK
RRRAKRRHHHHHHVDSSGRIVTD.

(2) INFORMATION FOR SEQ ID NO: 26:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 AMINO ACIDS
(B) TYPE:AMINO ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC2 TRANSPORT SEQUENCE

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:

(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26: (EX 13)

K R R R R K K R R Q R R R

(2) INFORMATION FOR SEQ ID NO: 27:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC2 OLIGONEUCLOTDE STRAND 1

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:

(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27: (EX 13)

AAG CGT CGA CGT AGA AAG AAA CGT AGA CAG CGT AGA CGT

(2) INFORMATION FOR SEQ ID NO: 28:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC 2 OLIGONUCLEOTIDE STRAND 2

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:

(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28: (EX 13)

TTC GCA GCT GCA TCT TTC TTT GCA TCT GTC GCA TCT GCA

(2) INFORMATION FOR SEQ ID NO: 29:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 783 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC2 cDNA

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29: (EX 13 DNA sequence)

GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA AAT ATT ATG TTA TTT ANA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT ANA GAT AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT AAA G AA TTC AAG CGT CGA CGT AGA AAG AAA CGT AGA CAG CGT AGA CGT CAC CAC CAC CAC CAC CAC GTC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA

(2) INFORMATION FOR SEQ ID NO: 30:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 260 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC2 PROTEIN

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:

(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30: (EX 13 Protein sequence

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVSYT
KSASEINGKLRQNKGVINGFPS
NLIKQVELLDKSFNKMKTPENIMLFXGDDPAYLGTEFQNTLLNSNGTINKTAFEKAKAKF
LNXDRLEYGYISTSLMNVSQ
FAGRPIITKFKVAKGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATM
MGTAINPKEFKRRRRKKRR
QRRRHHHHHHVDSSGRIVTD.

(2) INFORMATION FOR SEQ ID NO: 31:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC3 TRANSPORT PEPTIDE

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

RRKQRRKRR

(2) INFORMATION FOR SEQ ID NO: 32:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC3 OLIGONUCLEOTIDE STRAND 1

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32: (EX 14)

AGA AGG AAA CAA AGA AGA AAA AGA AGA

(2) INFORMATION FOR SEQ ID NO: 33:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC3 OLIGONUCLEOTIDE STRAND 2

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33: (EX 14)

TCT TCC TTT GTT TCT TCT TTT TCT TCT

(2) INFORMATION FOR SEQ ID NO: 34:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 771 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA C3BASIC3

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34: (EX 14 DNA sequence)

GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA AAT ATT ATG TTA TTT ANA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT ANA GAT AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT AAA GAA TTC AGA AGG AAA CAA AGA AGA AAA AGA AGA CAC CAC CAC CAC CAC CAC G TC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA

(2) INFORMATION FOR SEQ ID NO: 35:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 256 AMINO ACIDS
(B) TYPE: AMINO ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC3

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35: (EX 14)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVSYT
KSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPENIMLFXGDDPAYLGTEFQ
NTLLNSNGTINKTAFEKAKAKFLNXDRLEYGYISTSLMNVSQFAGRPIITKFKVAKGSKA

GYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFRRKQRRKR
RHHHHHHVDSSGRIVTD.

TITLE: FUSION PROTEINS

Field of the Invention

The present invention relates to conjugate or fusion type proteins comprising, for example, C3 (see below). Although, in the following, fusion-type proteins of the present invention, will be particularly discussed in relation to the use to facilitate generation of axons, it is to be understood that the fusion proteins may be exploited in other contexts.

The present invention in partcular pertains to the field of mammalian nervous system repair (e.g. repair of a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site), axon regeneration and axon sprouting.

The Rho family GTPases regulate axon growth and regeneration. Inactivation of Rho with Clostridium botulinum C3 exotransferase (hereinafter simply referred to as C3) can stimulate regeneration and sprouting of injured axons; C3 is a toxin obtainable from *Closteridium botulinum* (see Tigyi, et al. (1996)Journal of Neurochemistry.66:537-548, Jin and Strittmatter (1997) J. Neurosci.17:6256-6263). Compounds of the C3 family from *Closteridium botulinum* inactive Rho by ADP-ribosylation.

The present invention in particular relates to a means of delivery of C3 protein (e.g. C3 itself or other active analogs such as C3-like transferases - see below) or other Rho antagonists to repair damage in the nervous system. The means of delivery may take the form of chimeric (i.e. conjugate) C3-like Rho antagonists. These conjugate antagonists provide a significant improvement over C3 compounds (alone) because they are 3 to 4 orders of magnitude more potent with respect to the stimulation of axon growth on inhibitory substrates than recombinant C3 alone. Examples of these Rho antagonists have been made as recombinant proteins created to facilitate penetration of the cell membrane (i.e. to enhance cell uptake of the antagonists), improve dose-response when applied to neurons to stimulate growth, on growth inhibitory substrates, and to inactivate Rho. Examples of these conjugate Rho antagonists are described below in relation to the designations C3APL, C3APS, C3-TL, C3-

TS, C3-RTS, C3BASIC1 (a random, basic charge sequence added to the C-terminal of C3), C3BASIC2 (a random, basic charge sequence added to th C-terminal of C3) and C3BASIC3 (the reverse TAT sequence added to the C-terminal of C3).

Background

Traumatic injury of the spinal cord results in permanent functional impairment. Most of the deficits associated with spinal cord injury result from the loss of axons that are damaged in the central nervous system (CNS). Similarly, other diseases of the CNS are associated with axonal loss and retraction, such as stroke, HIV dementia, prion diseases, Parkinson's disease, Alzheimer's disease, multiple sclerosis and glaucoma. Common to all of these diseases is the loss of axonal connections with their targets, and the ability to stimulate growth of axons from the affected or diseased neuronal population would improve recovery of lost neurological functions. For example, following a white matter stroke, axons are damaged and lost, even though the neuronal cell bodies are alive. Treatments that are effective in eliciting sprouting from injured axons are equally effective in treating some types of stroke (Boston life sciences, Sept. 6, 2000 Press release). Similarly, although the the following discussion will generally relate to delivery of Rho antagonists, etc. to a traumatically damaged nervous system, this invention may also be applied to damage from unknown causes, such as during multiple sclerosis, HIV dementia, Parkinson's disease, Alzheimer's disease, prion diseases or other diseases of the CNS were axons are damaged in the CNS environment.

It has been proposed to use various Rho antagonists as agents to stimulate regeneration of (cut) axons, i.e. nerve lesions; please see, for example, Canadian Patent application nos. 2,304,981 (McKerracher et al) and 2,300,878 (Strittmatter). These patent application documents propose the use of known Rho antagonists such as for example C3, chimeric C3 proteins, etc. (see blow) as well as substances selected from among known trans-4-amino(alkyl)-1-pyridylcarbamoylcyclohexane compounds (also see below) or Rho kinase inhibitors for use in the regeneration of axons. C3 inactivates Rho by ADP-ribosylation and is fairly non-toxic to cells (Dillon and Feig (1995)Methods in Enzymology: Small GTPases and their regulators Part. B.256:174-184).

While the following discussion will generally relate or be directed at repair in the CNS, the techniques described herein may be extented to use in PNS repair. Treatment with Rho antagonists could be used to enhance the rate of axon growth in the PNS.

As mentioned above, traumatic injury of the spinal cord results in permanent functional impairment. Axon regeneration does not occur in the adult mammalian CNS because substrate-bound growth inhibitory proteins block axon growth. Many compounds, such as trophic factors, enhance neuronal differentiation and stimulate axon growth in tissue culture. However, most factors that enhance growth and differentiation are not able to promote axon regenerative growth on inhibitory substrates. To demonstrate that a compound known to stimulate axon growth in tissue culture most accurately reflects the potential for therapeutic use in axon regeneration in the CNS, it is important for the cell culture studies to include the demonstration that a compound can permit axon growth on growth inhibitory substrates. An example of trophic and differentiation factors that stimulate growth on permissive substrates in tissue culture, are neurotrophins such as nerve growth factor (NGF) and brain-derived growth factor. NGF, however, does not promote growth on inhibitory substrates (Lehmann, et al. (1999) 19: 7537-7547) and it has not been effective in promoting axon regeneration in vivo. BDNF is not effective to promote regeneration in vivo either (Mansour-Robaey, et al. (1994) 91: 1632-1636)

Targeting intracellular signalling mechanisms involving Rho and the Rho kinase promotes axon regeneration has been proposed (see, for example, the above mentioned Canadian Patent application nos. 2,304,981 (McKerracher et al)). For demonstration that inactivation of Rho promotes axon regeneration on growth inhibitory substrates, recombinant C3, a protein that inactivates by ADP ribosylation of the effector domain was used. While such a C3 protein can effectively promote regeneration, it has been noted that such a C3 protein does not easily penetrate into cells, and high doses must therefore be applied for it to be effective.

The high dose of recombinant C3 needed to promote functional recovery presents a practical

constraint or limitation on the use of C3 in vivo to promote regeneration (Lehmann, et al. (1999) 19: 7537-7547). In tissue culture studies, it has, for example, been determined that the minimum amount of C3 that can be used to induce growth on inhibitory substrates is 25 ug/ml. If the cells area not titurated, even this dose is ineffective (Figure 1). In the context of the present invention it has been determined, for example, that at least 40 ug/20 g mouse needs to be applied to injured mouse spinal cord or rat optic nerve. Calculating doses that would be required to treat an adult human on an equivalent dose per weight scale up used for our rat and mice experiments, it would be necessary to apply 120 mg/kg of C3 (i.e. alone) to the injured human spinal cord. This large of amount of recombinant protein creates significant problems for manufacturing, due to the large scale protein purification and cost. It also limits the dose ranging that can be tested because of the large amount of protein needed for minimal effective doses.

Another related limitation with respect to the use of C3 to promote repair in the injured CNS is that it does not easily penetrate the plasma membrane of living cells. In tissue culture studies when C3 is applied to test biological effects it has been microinjected directly into the cell (Ridley and Hall (1992) 70: 389-399), or applied by trituration of the cells to break the plasma membrane (Lehmann, et al. (1999) 19: 7537-7547, Jin and Strittmatter (1997) 17: 6256-6263). In the case of axon injury in vivo, the C3 protein is likely able to enter the cell because injured axons readily take up substances from their environment.

Summary of the invention

The term "Rho antagonists" as used herein includes, but is not restricted to, (known) C3, including C3 chimeric proteins, and like Rho antogonists .

The term "nerve injury site" refers to a site of traumatic nerve injury or nerve injury caused by disease. The nerve injury site may be a single nerve (eg sciatic nerve) or a nerve tract comprised of many nerves (eg. damaged region of the spinal cord). The nerve injury site may be in the central nervous system of peripheral nervous system in any region needing repair. The nerve injury site may form as a result of damage caused by stroke. The nerve injury site may be in the

brain as a result of surgery, brain tumour removal or therapy following a cancerous lesion. The nerve injury site may result from Parkinson's disease, Alzheimer's disease, Amyotrophic lateral sclerosis, diabetes or any other type of neurodegenerative disease.

The term "pharmaceutically acceptable carrier" or "adjuvant" and "physiologically acceptable vehicle" and the like are to be understood as referring to an acceptable carrier or adjuvant that may be administered to a patient, together with a compound of this invention, and which does not destroy the pharmacological activity thereof.

It is to be understood herein, that if a "range" or "group of substances" is mentioned with respect to a particular characteristic (e.g. amino acid groups, temperature, pressure, time and the like) of the present invention, the present invention relates to and explicitly incorporates herein each and every specific member and combination of sub-ranges or sub-groups therein whatsoever. Thus, any specified range or group is to be understood as a shorthand way of referring to each and every member of a range or group individually as well as each and every possible sub-ranges or sub-groups encompassed therein; and similarly with respect to any sub-ranges or sub-groups therein. Thus, for example,

- with respect to a sequence comprising up to 50 base units it is to be understood as specifically incorporating herein each and every individual unit, as well as sub-range of units;

- and similarly with respect to other parameters such as low pressures, concentrations, elements, etc...

It is also to be understood herein that "g" or "gm" is a reference to the gram weight unit; that "C" is a reference to the celsius temperature unit; and "psig" is a reference to "pounds per square inch guage".

In accordance with the present invention a conjugate or fusion protein comprising a therapeutically active agent is provided whereby the active agent may be delivered across a cell wall membrane, the conjugate or fusion protein comprising a transport subdomain(s) or moiety(ies) in addition to an active agent moiety(ies). More particularly, as discussed herein, in acccordance with the present invention a conjugate or fusion protein is provided wherein the therapeutically active agent is one able to faciliate (for facilitating) axon growth (e.g. regeneration) i.e. a conjugate or fusion proteinin the form of a conjugate Rho antagonist.

The present invention in acccordance with an aspect thereof provides a drug delivery construct or conjugate [e.g. able to (for) suppress(ing) the inhibition of neuronal axon growth at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site] comprising at least one transport agent region and an active agent region not naturally associated with the active agent region, wherein the transport agent region is able to facilitate (i.e. facilitates) the uptake of the active agent region into a mammalian (i.e. human or animal) tissue or cell, and wherein the active agent region is an active therapeutic agent region able (i.e. has the capacity or capability) to facilitate axon growth (e.g. regeneration) , including a derivative or homologue thereof (i.e. pharmaceutically acceptable chemical equivalents thereof - pharmaceutically acceptable derivative or homologue).

In accordance with the present invention the active agent region may be an ADP-ribosyl transferase C3 region. In accordance with the present invention the ADP-ribosyl transferase C3 may be selected from the group consisting of ADP-ribosyl transferase derived from *Closteridum botulinum* and a recombinat ADP-ribosyl transferase.

In accordance with another aspect the present invention provides a drug conjugate consisting of a transport polypeptide moiety (e.g. rich in base pairs e.g. 50 base pairs or more) covalently linked to an active cargo moiety (e.g. by a labile bond (i.e. a bond readily cleavable or subject to chemical change in the interior target cell environment)) wherein the transport polypeptide moiety is able to or has the capability to facilitate(s) the uptake of the active cargo moiety into

a mammalian (e.g. human or animal) tissue or cell (for example, a transport subdomain of HIV Tat protein, a transport homeoprotein (e.g. the homeodomain of antennopedia), or a variation derivative or homolog thereof, (i.e. pharmaceutically acceptable chemical equivalents thereof)) [by a receptor independent processses] and wherein the active cargo moiety is an active therapeutic moiety able (i.e. has the capacity or capability) to facilitate (i.e. for facilitating) axon growth (e.g. regeneration).

In acccordance with the present nvention the transport polypeptide moiety may be selected from the group consisting of a transport subdomain of HIV Tat protein, the homeodomain of antennopedia, and a functional derivative and analog thereof [i.e. by the adddition of polyamine, or any random sequence enriched in basic aminoacids] - [i.e. pharmaceutically acceptable chemical equivalents thereof] and wherein the active cargo moiety is selected from the group consisting of C3 protein able (i.e. has the capacity or capability) to facilitate (i.e. for facilitating) axon growth (e.g. regeneration).

In acccordance with the present invention the C3 protein may be ADP-ribosyl transferase C3. In accordance with the present invention the ADP-ribosyl transferase C3 imay be selected from the group consisting of ADP-ribosyl transferase derived from *Closteridum botulinum* and a recombinat ADP-ribosyl transferase. In accordance with the present invention the transport polypeptide moiety may include an active contiguous amine acid sequence as described herein

In accordance with an additional aspect the present invention provides a fusion protein [e.g. able to (for) suppress(ing) the inhibition of neuronal axon growth at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site] consisting of a carboxy terminal active cargo moiety and an amino terminal transport moiety, wherein the terminal transport moiety is selected from the group consisting of a transport subdomain of HIV Tat protein, a transport homeoprotein (e.g. the homeodomain of antennopedia), and a functional derivatives and analogs thereof (i.e. pharmaceutically acceptable chemical equivalents thereof) and wherein the active cargo moiety consists of a C3 protein.

In accordance with the presetn invention the C3 protein may be ADP-ribosyl transferase C3. In accordance with the presetn invention the ADP-ribosyl transferase C3 is selected from the group consisting of ADP-ribosyl transferase derived from *Closteridum botulinum* and a recombinat ADP-ribosyl transferase.

The present invention in particular provides a fusion protein (e.g. able to (for) suppressing the inhibition of neuronal axon growth at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site) consisting of a carboxy terminal active cargo moiety and an amino terminal transport moiety, wherein the terminal transport moiety consists of the homeodomain of antennopedia and the active cargo moiety consists of a C3 protein (i.e. as described herein).

The present invention also in particular provides a fusion protein (e.g. able to (for) suppressing the inhibition of neuronal axon growth at a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site) consisting of a carboxy terminal active cargo moiety and an amino terminal transport moiety, wherein the terminal transport moiety consists of a transport subdomain of HIV Tat protein and the active cargo moiety consists of a C3 protein (i.e. as descried herein).

The present invention in a furthe aspect provides for the use of a member selected from the group consisting of a drug delivery construct as described herein, a drug conjugate as described herein and a fusion protein as described herein (e.g. including pharmaceutically acceptable chemical equivalents thereof) for suppressing the inhibition of neuronal axon growth.

The present invention a pharmaceutical composition (e.g. for suppressing the inhibition of neuronal axon growth), the pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and an effective amount of an active member selected from the group consisting of a drug delivery construct as described herein, a drug conjugate as described

herein, and a fusion protein as described herein (e.g. including pharmaceutically acceptable chemical equivalents thereof).

The present invention further provides for the use of a member selected from the group consisting of a drug delivery construct as described herein, a drug conjugate as described herein, and a fusion protein as described herein (e.g. including pharmaceutically acceptable chemical equivalents thereof) for the manufacture of a pharmaceutical composition (e.g. for suppressing the inhibition of neuronal axon growth).

The present invention also relates to a methd for preparing a conjugate or fusion protein as defined above comprising

- cultivating a host cell under conditions hich provide for the expression of the conjugate or fusion protein within the cell ; and

- recovering the conjugate or fusion protein by affinity purification under non-denaturing conditions.

The present invention in particular provides a fusion protein selected from the group consisting of C3APL , C3APS, C3-TL, C3-TS C3-RTS, C3BASIC2 and C3BASIC3 and pharmaceutically acceptable chemical equivalents thereof.

The present invention in another aspect provides a method of suppressing the inhibition of neuronal axon growth comprising administering (e.g.delivering) a member selected from the group consisting of a drug delivery construct as described herein, a drug conjugate as described herein and a fusion protein as described herein (e.g. including pharmaceutically acceptable chemical equivalents thereof) (e.g. directly) to a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site (of a patient), in an amount effective to counteract said inhibition.

The present invention, for example, provides recombinant Rho antagonists comprising C3 enzymes with basic stretches of amino acids added to the C3 coding sequence to facilitate the uptake thereof into tissue or cells for the repair and/or promotion of repair in the CNS, even in the lack of traumatic axon damage.

The invention in particular provides C3 proteins, which may have additional amino acids added to the carboxy terminal end of the C3 proteins. Examples of such proteins includes:

C3APL: (C3 antennapedia –long) created by annealing sequences from the antennapedia transcription factor to the 5' end of the sequence encoding C3 cDNA. The long antennapedia sequence of 60 amino acids containing the homeodomain of antennapedia, was used;

C3APS: A short 11 amino acid sequence of antennapedia that has transmembrane transport properties was fused to the carboxy terminal of C3 to create C3APS;

C3-TL: C3 tat-long created by fusing amino acids 27 to 72 of the carboxy terminal of C3 protein.

C3-TS: C3 tat-short created by fusing the amino acids YGRKRRQRRR to the C3 protein; and

C3-T RS: C3 tat-short created by fusing the amino acids RRQRRKKR to the C3 protein.

It has been found that conjugate or fusion protein antagonists of the present invention are effective to stimulate repair in the CNS after spinal cord injury. It is obvious that the cell permeability would now allow treatment of victims of stoke and neurodegenerative disease with the new antagonists because Rho signalling pathway is important in repair after stroke (Hitomi, et al. (2000) 67: 1929-39.). Treatment with Rho antagonists in the adhesive delivery system could be used to enhance the rate of axon growth in the PNS. Also, evidence in the literature now links Rho signalling with formation of Alzheimer's disease tangles through its ability to activate PKN which then phosphorylates tau and neurofilaments (Morissette, et al. (2000) 278: H1769-74., Kawamata, et al. (1998) 18: 7402-10., Amano, et al. (1996) 271: 648-50., Watanabe, et al. (1996) 271: 645-8.). Therefore, Rho antagonists are expected to be useful in the treatment of

Alzheimer's disease. The new chimeric C3 drugs should are able to diffuse readily and therefore can promote repair for diseases that are neurodegenerative. Examples include, but are not limited to stroke, traumatic brain injury, Parkinson's disease, Alzheimer's disease and ALS. Moreover, it is now well established that Rho signalling antagonists are effective in the treatment of diseases. These include, but are not limited to eye diseases such as glaucoma (Honjo, et al. (2001) 42: 137-44., Rao, et al. (2001) 42: 1029-1037.), cancer cell migration and metastasis (Sahai, et al. (1999) 9: 136-45., Takamura, et al. (2001) 33: 577-81., Imamura, et al. (2000) 91: 811-6.). The effect of the Rho signalling pathway on smooth muscle relaxation are well established. This has led to the identification of Rho signalling antagonists as effective in treatment of hypertension (Chitaley, et al. (2001) 3: 139-144., Somlyo (1997) 389: 908-911, Uehata, et al. (1997) 389: 990-994) , asthma (Nakahara, et al. (2000) 389: 103-6., Ishizaki, et al. (2000) 57: 976-83), and vascular disease (Miyata, et al. (2000) 20: 2351-8., Robertson, et al. (2000) 131: 5-9.)as well as penile erectile dysfunction (Chitaley, et al. (2001) 7: 119-22.)

Rho GTPases include members of the Rho, Rac and Cdc42 family of proteins. Our invention concerns Rho family members of the Rho class. Rho proteins consist of different variants encoded by different genes. For example, PC12 cells express RhoA, RhoB and RhoC (Lehmann et al 1999 IBID); PC12 cells: Pheochromocytom cell ligne (Greene A and Tischler, A S PNAS 73:2424 (1976). To inactivate Rho proteins inside cells, Rho antagonists of the C3 family type are effective because they inactivate all forms of Rho (eg. RhoA, Rho B etc). In contrast, gene therapy techniques, such as introduction of a domainant negative RhoA family member into a diseased cell, will only inactivate that specific RhoA family member.

Recombinant C3 proteins, or C3 proteins that retain the ribosylation activity are also effective in our delivery system and are covered by this invention. In addition, Rho kinase is a well-known target for active Rho, and inactivating Rho kinase has the same effect as inactiving Rho, at least in terms of neurite or axon growth (Kimura and Schubert (1992)Journal of Cell Biology.116:777-783, Keino-Masu, et al. (1996)Cell.87:175-185, Matsui, et al. (1996)EMBO J.15:2208-2216, Matsui, et al. (1998)J. Cell Biol.140:647-657, Ishizaki (1997)FEBS Lett.404:118-124), the biological activity that concerns this invention

The C3 polypeptides of the present invention include biologically active fragments and analogs of C3; fragments encompass amino acid sequences having truncations of one or more amino acids , wherein the truncation may originate from the amino terminus, carboxy terminus, or from the interior of the protein. Analogs of the invention involve an insertion or a substitution of one or more amino acids.. Fragments and analogs will have the biological property of C3 that is capable of inactivation Rho GTPases. Also encompassed by the invention are chimeric polypeptides comprising C3 amino acid sequences fused to heterologous amino acid sequences. Said heterologous sequences encompass those which, when formed into a chimera with C3 retain one or more biological or immunological properties of C3. A host cell transformed or transfected with nucleic acids encoding C3 protein or c3 chimeric protein are also encompassed by the invention. Any host cell which produces a polypeptide having at least one of the biological properties of a C3 may be used. Specific examples include bacterial, yeast, plant, insect or mammalian cells. In addition, C3 protein may be produced in transgenic animals. Transformed or transfected host cells and transgenic animals are obtained using materials and methods that are routinely available to one skilled in the art. Host cells may contain nucleic acid sequences having the full-length gene for C3 protein including a leader sequence and a C-terminal membrane anchor sequence (see below) or, alternatively, may contain nucleic acid sequences lacking one or both of the leader sequence and the C-terminal membrane anchor sequence. In addition, nucleic acid fragments, variants and analogs which encode a polypeptide capable of retaining the biological activity of C3 may also be resident in host expression systems.

The Rho antogaonist that is a recombinant proteins can be made according to methods present in the art. The proteins of the present invention may be prepared from bacterial cell extracts, or through the use of recombinant techniques. In general, C3 proteins according to the invention can be produced by transformation (transfection, transduction, or infection) of a host cell with all or part of a C3-encoding DNA fragment in a suitable expression vehicle. Suitable expression vehicles include: plasmids, viral particles, and phage. For insect cells, baculovirus expression vectors are suitable. The entire expression vehicle, or a part thereof, can be integrated into the host cell genome. In some circumstances, it is desirable to employ an inducible expression vector.

Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems can be used to provide the recombinant protein. The precise host cell used is not critical to the invention. The C3 protein can be produced in a prokaryotic host (e.g., E. coli or B. subtilis) or in a eukaryotic host (e.g., Saccharomyces or Pichia; mammalian cells, e.g., COS, NIH 3T3, CHO, BHK, 293, or HeLa cells; or insect cells).

Proteins and polypeptides can also be produced by plant cells. For plant cells viral expression vectors (e.g., cauliflower mosaic virus and tobacco mosaic virus) and plasmid expression vectors (e.g., Ti plasmid) are suitable. Such cells are available from a wide range of sources (e.g., the American Type Culture Collection, Rockland, Md.). The methods of transformation or transfection and the choice of expression vehicle will depend on the host system selected.

The host cells harbouring the expression vehicle can be cultured in conventional nutrient media adapted as need for activation of a chosen gene, repression of a chosen gene, selection of transformants, or amplification of a chosen gene. One expression system is the mouse 3T3 fibroblast host cell transfected with a pMAMneo expression vector (Clontech, Palo Alto, Calif.). pMAMneo provides an RSV-LTR enhancer linked to a dexamethasone-inducible MMTV-LTR promotor, an SV40 origin of replication which allows replication in mammalian systems, a selectable neomycin gene, and SV40 splicing and polyadenylation sites. DNA encoding a C3 protein would be inserted into the pMAMneo vector in an orientation designed to allow expression. The recombinant C3 protein would be isolated as described below. Other preferable host cells that can be used in conjunction with the pMAMneo expression vehicle include COS cells and CHO cells (ATCC Accession Nos. CRL 1650 and CCL 61, respectively).

C3 polypeptides can be produced as fusion proteins. For example, expression vectors can be used to create lacZ fusion proteins. The pGEX vectors can be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can be easily purified from lysed cells by adsorption to

glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety. Another stategy to make fusion proteins is to use the His tag system.

In an insect cell expression system, Autographa californica nuclear polyhedrosis virus AcNPV), which grows in Spodoptera frugiperda cells, is used as a vector to express foreign genes. A C3 coding sequence can be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter, e.g., the polyhedrin promoter. Successful insertion of a gene encoding a C3 polypeptide or protein will result in inactivation of the polyhedrin gene and production of non-occluded recombinant virus (i.e., virus lacking the proteinaceous coat encoded by the polyhedrin gene). These recombinant viruses are then used to infect spodoptera frugiperda cells in which the inserted gene is expressed (see, Lehmann et al for an example of making recombinant MAG protein).

In mammalian host cells, a number of viral-based expression systems can be utilised. In cases where an adenovirus is used as an expression vector, the C3 nucleic acid sequence can be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene can then be inserted into the adenovirus genome by in vitro or in vivo recombination. Insertion into a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing a C3 gene product in infected hosts.

Specific initiation signals may also be required for efficient translation of inserted nucleic acid sequences. These signals include the ATG initiation codon and adjacent sequences. In cases where an entire native C3 gene or cDNA, including its own initiation codon and adjacent sequences, is inserted into the appropriate expression vector, no additional translational control signals may be needed. In other cases, exogenous translational control

signals, including, perhaps, the ATG initiation codon, must be provided. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators.

In addition, a host cell may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in a specific, desired fashion. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product can be used. Such mammalian host cells include, but are not limited to, CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and in particular, choroid plexus cell lines.

Alternatively, a C3 protein can be produced by a stably-transfected mammalian cell line. A number of vectors suitable for stable transfection of mammalian cells are available to the public; methods for constructing such cell lines are also publicly available. In one example, cDNA encoding the C3 protein can be cloned into an expression vector that includes the dihydrofolate reductase (DHFR) gene. Integration of the plasmid and, therefore, the C3 protein-encoding gene into the host cell chromosome is selected for by including 0.01-300 μM methotrexate in the cell culture medium (as described in Ausubel et al., supra). This dominant selection can be accomplished in most cell types.

Recombinant protein expression can be increased by DHFR-mediated amplification of the

transfected gene. Methods for selecting cell lines bearing gene amplifications are known in the art; such methods generally involve extended culture in medium containing gradually increasing levels of methotrexate. DHFR-containing expression vectors commonly used for this purpose include pCVSEII-DHFR and pAdD26SV(A). Any of the host cells described above or, preferably, a DHFR-deficient CHO cell ligne (e.g., CHO DHFR cells, ATCC Accession No. CRL 9096) are among the host cells preferred for DHFR selection of a stably-transfected cell line or DHFR-mediated gene amplification.

A number of other selection systems can be used, including but not limited to the herpes simplex virus thymidine kinase, hypoxanthine-guanine phosphoribosyltransferase, and adenine phosphoribosyltransferase genes can be employed in tk, hgprt, or aprt cells, respectively. In addition, gpt, which confers resistance to mycophenolic acid ; neo, which confers resistance to the aminoglycoside G-418; and hygro, which confers resistance to hygromycin. can be used.

Alternatively, any fusion protein can be readily purified by utilising an antibody specific for the fusion protein being expressed. For example, a system described in Janknecht et al. (1981) Proc. Natl. Acad. Sci. USA 88, 8972, allows for the ready purification of non-denatured fusion proteins expressed in human cell lines. In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the gene's open reading frame is translationally fused to an amino-terminal tag consisting of six histidine residues. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni2+ nitriloacetic acid-agarose columns, and histidine-tagged proteins are selectively eluted with imidazole-containing buffers.

Alternatively, C3 or a portion thereof, can be fused to an immunoglobulin Fc domain. Such a fusion protein can be readily purified using a protein A column.

To test Rho antagonists for activity, a tissue culture bioassay system was used. This bioassay is used to define activity of Rho antagonists that will be effective in promoting axon regeneration in spinal cord injury, stroke or neurodegenerative disease.

Neurons do not grow neurites on inhibitory myelin substrates. When neurons are placed on inhibitory substrates in tissue culture, they remain rounded. When an effective Rho antagonist is added, the neurons are able to grow neurites on myelin substrates. The time that it takes for neurons to growth neurites upon the addition of a Rho antagonist is the same as if neurons had been plated on growth permissive substrate such as laminin or polylysine, typically 1 to 2 days in cell culture. The results can be scored visually. If needed, a quantitative assessment of neurite growth can be performed. This involved measuring the neurite length in a) control cultures where neurons are plated on myelin substrates and left untreated b) in positive control cultures, such as neurons plated on polylysine c) or treating cultures with different concentrations of the test antagonist.

To test C3 in tissue culture, it has been found that the best concentration is 25-50 ug/ml. Thus, high concentrations of this Rho antagonist are needed as compared to the growth factors used to stimulate neurite outgrowth. Growth factors, such as nerve growth factor (NGF) are used at concentrations of 1- 100 ng/ml in tissue culture. However, growth factors are not able to overcome growth inhibition by myelin. Our tissue culture experiments are all performed in the presence of the growth factor BDNF for retinal ganglion cells, or NGF for PC12 cells. When growth factors have been tested in vivo, typically the highest concentrations possible are used, in the ug/ml range. Also they are often added to the CNS with the use of pumps for prolonged delivery (eg. Ramer et al, IBID). For in vivo experiments the highest concentrations possible was used when working with C3 stored as a frozen 1 mg/ml solution.

The Rho antagonist C3 is stable at 37 C for at least 24 hours. The stability of C3 was tested in tissue culture with the following experiment. The C3 was diluted in tissue culture medium,

left in the incubator at 37C for 24 hours, then added to the bioassay system described above, using retinal ganglion cells as the test cell type. These cells were able to extend neurites on inhibitory substrates when treated with C3 stored for 24 hours at 37C. Therefore , the minimun stability is 24 hours. This is in keeping with the stability projection based on amino acid composition (see sequence data, below).

A compound can be confirmed as a Rho antagonist in one of the following ways:

a) Cells are cultured on a growth inhibitory substrate as above, and exposed to the candidate Rho antagonist;

b) Cells of step a) are homogenized and a pull-down assay is performed. This assay is based on the capability of GST-Rhotektin to bind to GTP-bound Rho. Recombinant GST-Rhotektin or GST rhotektin binding domain (GST-RBD) is added to the cell homogenate made from cells cultured as ina). It has been found that inhibitory substrates activate Rho, and that this activated Rho is pulled down by(GST-RBD). Rho antagonists will block activation of Rho, and therefore, an effective Rho antagonist will block the detection of Rho when cell are cultured as described by a) above;

c) An alternate method for this pull-down assay would be to use the GTPase activating protein, Rho-GAP as bait in the assay to pull down activated Rho, as described (Diekmann and Hall, 1995. In Methods in Enzymology Vol. 256 part B 207-215).

Another method to confirm that a compound is a Rho antagonist is as follows:

When added to living cells antagonists that inactivate Rho by ADP-ribosylation of the effector domain can be identified by detecting a molecular weight shift in Rho (Lehmann et al, 1999 Ibid). The molecular weight shift can be detected after treatment of cells with Rho antagonist by homogenizing the cells, separating the proteins in the cellular homogenate by SDS polyacrylamide gel electrophoresis. The proteins are transferred to nitrocellulose paper, then Rho is detected with Rho-specific antibodies by a Western blotting technique.

Another method to confirm that compound is a Rho-kinase antagonist is as follows:

a) Recombinant Rho kinase tagged with myc epitope tag, or a GST tag is expressed in Hela cells or another suitable cell type by transfection.

b) The kinase is purified from cell homogenates by immunoprecipation using antibodies directed against the myc tag or the GST tag.

c) The recovered immunoprecipitates from b) are incubated with [32P] ATP and histone type 2 as a substrate in the presence or absence of the Rho kinase. In the absence of Rho kinase activity the Rho kinase antigens is able to block the phosphorylation activity of Rho kinase (i.e. phosphorylation of hislore), and as such identified the compound as a Rho kinase antagonist.

Turning now to the trasport side of the conjugates of the present invention , known methods are available to add transport sequences that allow proteins to penetrate into the cell; examples include membrane translocating sequence (Rojas (1998) 16: 370-375), Tat-mediated protein delivery (Vives (1997) 272: 16010-16017), polyargine sequences (Wender et al. 2000, PNAS 24: 13003-13008) and antennapedia (Derossi (1996) 271: 18188-18193). Examples of known tranport agents, moities, subdomains and the like are also shown for example in Canadian patent document no. 2,301,157 (conjugates containing homeodomain of antennopedia) as well as in U.S. patent 5,652,122, 5,670,617, 5,674,980, 5,747,641, and 5,804604 (conjugates containing amino acids of Tat HIV protein (hereinafter Tat HIV protein is sometimes simply referred to as Tat); the entire contents of each of these patent documents is incorporated herein by reference.

Several receptor –mediated transport strategies have been used to try and improve function of ADP ribosylases: these methods include fusing C2 and C3 sequences ENRfu(Wilde, et al. (2001) 276: 9537-9542.) and use of receptor-mediated transport with the diptheria toxin receptorENRfu(Aullo, et al. (1993) 12: 921-31.). These methods have not been demonstrated to dramatically increase the potency of C3. Moreover, these proteins require receptor-

mediated transport. This means that the cells must express the receptor, and must express sufficient quantities of the receptor to significantly improve transport. In the case of dipthera toxin, not all cells express the appropriate receptor, limiting its potential use. The clinical importance for any of these has not been tested or shown.

One strategy which may be used in accordance with the present invention is to exploit the antennapedia homeodomain that is able to transport proteins across the plasma membrane by a receptor-independent mechanism (Derossi (1996) 271: 18188-18193); an alternate strategy is to exploit tat-mediated delivery (Vives (1997) 272: 16010-16017, Fawell (1994) 91: 664-668, Frankel (1988) 55: 1189-1193).

The Anntenapedia strategy has been used for protein translocation into neurons (Derossi (1996) 271: 18188-18193). Anntenapedia has, for examle, been used to transport biotin-labelled peptides in order to demonstrate the efficacy of the technique; see U.S. Patent no. 6,080,724 (the entire contents of this patent re incorporated herein by reference). Antennapedia enhances growth and branching of neurons in vitro (Bloch-Gallego (1993) 120: 485-492). Homeoproteins are transcription factors that regulate development of body organization, and antennapedia is a *Drosophila* homeoprotein. Tat on the other hand is a regulatory protein from human immunodeficiency virus (HIV) that is expressed in the long terminal repeat. It is a highly basic protein that is found in the nucleus and can transport reported genes into cell. Moreover, tat-linked proteins can penetrate cells after intraperotoneal injection, and it can even cross the blood brain barrier to enter cells within the brain (Schwarze, et al. (1999) 285: 1569-72).

In the context of axon growth on inhibitory substrates, axon regeneration after injury, or axon regeneration in the brain or spinal cord, no method using these transport sequences has been devised. In particular, it should be noted that the ability of antennapedia to enhance growth was tested with neurons placed on laminin-coated coverslips. Laminin supports axon growth and overrides growth inhibition (David, et al. (1995) 42: 594-602) thus, it is not a suitable

substrate to test the potential for regeneration. There is an enormous wealth of literature over the last 20 years on substances that promote axon growth under such favourable tissue culture conditions, but none of these has lead to clinical advances in the treatment of spinal cord injury. The effect of antennapedia was shown to act as similar to a growth factors. Growth factors do not over come growth inhibition by CNS growth inhibitory substrates (Lehmann, et al. (1999) 19: 7537-7547, Cai, et al. (1999) 22: 89-101). Growth factors applied in vivo do not support regeneration, only sprouting (Schnell, et al. (1994) 367: 170-173).

The transport sequence may be added to the n-terminal sequence of the C3 protein. Aternatively, the transport sequence may be added on the C-terminal end of the C3 protein; because the C-terminal is already quite basic, this should enhance further the transport properties

The new chimeric C3 may be used to treat spinal cord injury to promote functional repair. We have demonstrated that both C3APL and C3APS can overcome growth inhibition on complex inhibitory substrates that include myelin and mixed condroitin sulfate proteoglycans. Further, we demonstrate that C3APL can promote functional recovery after application to injured spinal cord in adult mice. The new chimeric protein may be used to promote axon regeneration and reduce scarring after CNS injury. Scarring is a barrier to nerve regeneration.

The advantage of the new chimeric C3 is that ability to treat the injured axons after a significant delay between the injury and the treatment. Also, the new recombinant protein may be useful in the treatment of chronic injury. The chimeric C3 can also be used to treat neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease where penetration of the Rho antagonist to the affected neuronal population is required for effective treatment. The chimeric C3 will also be of benefit for the treatment of stroke and traumatic brain injury. Moreover, much evidence suggests efficacy in the treatment of cancer cell migration. Rho antagonists are also useful in the treatment of disease involving smooth muscle, such as vascular disease, hypertention, asthma, and penile dysfunction.

For treatment of spinal cord injury, the conjugate Rho antagonists of the present invention may be used in conjunction cell transplantation. Many different cell transplants have been extensively tested for their potential to promote regeneration and repair, including , but a not restricted to, Schwann cells, fibroblasts modified to express growth factors, fetal spinal cord transplants, macrophages, embryonic or adult stem cells, and olfactory ensheathing glia. C3APL and C3APshort may be used in conjunction with neurotrophins, apoptosis inhibitors, or other agents that prevent cell death. They may be used in conjunction with cell adhesion molecules such as L1, laminin, and artifical growth matrices that promote axon growth. The chimeric C3 constructs of the present invention may also be used in conjunction with the use of antibodies that block growth inhibitory protein substrates to promote axon growth. Examples of such antibody methods are the use of IN-1 or related antibodies (Schnell and Schwab (1990) 343: 269-272)or through the use of therapeutic vaccine approaches (Huang (1999) 24: 639-647).

BRIEF DESCRIPTION OF THE FIGURES

In drawings which llustrate example embodiments of the present invention:

Figure 1 illustrates the Dose response of normal C3 with and without trituration;

Figure 2 illustrates ADP ribosyation by C3AL and C3APS, but not C3 after passively adding the comppounds to PC12 cells;

Figure 3A illustrates that C3APL penetrates cells;

Figure 3B illustrates a lower level of cell peneration by C3 as compared to figure 3A;

Figure 4 illustrates the effectiveness of C3APL and C3APS at low doses;

Figure 5 illustrates the effectiveness of C3APL and C3APS at low doses;

Figure 6 illustrates the effeciveness of CAPL to stimulate axon regeneration of primary neurons;

Figure 7 illustrates the effectiveness of C3APL to promore functional recovery ater spinal cors injury;

Figure 8 illustrates effectiveness of TAT transport sequences to enhance growth as C3-TAT chimeras; and

Figures 9A and 9B illustrate axon regeneration after spinal cord injury and treatment with C3APL.

Referring to figure 1, PC12 cells were plated on inhibitory myelin substrates. Unmodified C3 added to the tissue culture medium at concentration from 0.00025 – 50 ug/ml did not significantly improve neurite outgrowth over the untreated control (0). C3 was only effective n stimulating neurite outgrowth for cells plated on myelin substrates after scrape –loading. This figure demonstrates the limited or no penetration in cells when passively added to the tissue culture medium. Please see example 4 below for techniques.

Referring to figure 2 this figure provides a demonstration of the new C3 ribosylated Rho C3APL and C3APS ADP ribosylate Rho. Western blot of showing RhoA in untreated cells (lane 1), and cells treated with C3APL (lane 2) or C3APS (lane 3). When Rho is ADP ribosylated by C3 it undergoes a molecular weight shift, as observed for lanes 2 and 3. Please see example 4 below for techniques.

Referring to figure 3 this figure shows intracellular activty after treatment with C3APL.

Detection that the new fusion C3 penetrates into the cell wash. Immunocytochemistry with anti-C3 antibody of PC12 cells plated on myelin and treated with C3 (A) or C3APL (B). Cells in A (figure 3A) are not immuoreactive because C3 has not penetrated into the cells. Cells in B (figure B) are immunoreactive and they are able to extend neurites on myelin substrates. Please see example 4 below for techniques.

Turnng to figure 4 it shows C3-antennopedia proteins promote growth on ihibitory substrates. The dose response experiment shows that C3APL and C3APS promote more neurite growth per cell than control PC12 cells plated on myelin. PC12 cells were plated on myelin and either scrape loaded with unmodified C3 (C3 50) left untreated (0) or treated with various concentrations of C3APL. Compared to C3 used at 25 ug/ml, C3APS is effective at stimulating more cells to grow neurites at 0.0025 ug/ml, a dose 10,000 X less. Please see example 4 below for techniques.

Figure 5 shows a dose response experiment showing that C3APL and C3APS elicit long neurites to grow when cells are plated on inhibitory substrates. PC12 cells were plated on myelin and either scrape loaded with unmodified C3 (C3 50) left untreated (0) or treated with various concentrations of C3APL. Compared to C3 used at 25 ug/ml, C3APS is effective at stimulating more cells to longer neurite growth at 0.0025 ug/ml, a dose 10,000 X less. Please see example 4 below for techiniques.

As may be seen in figure 6 shows primary neurons growing on inhibitory substrates after treatment with C3APL. Rat retinal ganglion cells were plated on myelin substrates and treated with different concentrations of C3APL. Concentrations of 0.025 and above promoted significantly longer neurites. This dose is 1000X lower than that of C3 need to promote growth on myelin.

Referring to figure 7 this figure shows behavioral recovery after treatment of adult mice with C3APL. Dose-response experiment. Mice received a dorsal hemisection of the spinal cord and were left untreated (transection), were treated with fibrin alone (fibrin) or were treated with fibrin plus C3 at the indicated concentrations given in ug/mouse. Each point represents one animal. The BBB score was assessed 24 hours after treatment. Animals treated with C3 exhibed a significant improvement in behavioural recovery than untreated animals. The effective dose of 0.5 ug is 100X less than unmodified C3 used. Please see example 6.

Referrring to figure 8 tis figure shows promotion of axon growth by TAT-C3 chimeric proteins. The dose response experiment shows that C3-TS and C3-TL promote more neurite growth per cell than control PC12 cells plated on myelin. PC12 cells were plated on myelin and either scrape loaded with unmodified C3 (scrape load) left untreated (myelin) or treated with various concentrations of C3-TS (grey bars) or C3-TL (black bars). Compared to C3 used at 25 ug/ml, C3-TL is effective at stimulating more cells to grow neurites at 0.0025 ug/ml, a dose 10,000 X less than C3.

Refering to figure 9A and 9B these figures show axon regeneration in injured spinal cord, i.e. anatomical regeneration after treatment with C3APL. Section of the spinal cord after anterograde labeling with WGA HRP. A) Sprouting of cut axons in to the dorsal white matter. Arrows show regenerating axons distal to the lesion. B) Same section 3 mm from the lesion site. Arrows show regenerating axons

DETAILED DESCRIPTION

Method for making the C3APL and C3AP-short proteins

C3APL is the name given to the protein made by ligating a cDNA encoding C3 (Dillon and Feig (1995) 256: 174-184) with cDNA encoding the antennapedia homeodomain (Bloch-Gallego (1993) 120: 485-492). The stop codon at the 3' end of the DNA was replaced with an EcoR1 site by polymerase chain reaction using the primers 5'GAA TTC TTT AGG ATT GAT AGC TGT GCC 3' (SEQ ID NO: 1) and 5'GGT GGC GAC CAT CCT CCA AAA 3' (SEQ ID NO: 2). The PCR product was sub-cloned into a pSTBlue-1 vector (Novagen, city), then cloned into a pGEX-4T vector using BamH I and Not I restriction site. This vector was called pGEX-4T/C3. The antennapedia sequence used to add to the 3' end of C3 in pGEX-4T/C3 was created by PCR from the pET-3a vector (Bloch-Gallego (1993) 120: 485-492, Derossi (1994) 269: 10444-10450), subcloned into a pSTBlue-1 blunt vector,

then cloned into the pGEX-4T/C3, using the restriction sites EcoR I and Sal I, creating pGEX-4T/C3APL

A shorter version of the Antennapedia (pGEX-4T/C3APS) was also made. This chimeric sequence was made by ligating oligonucleotides encoding the short antennapedia peptide (Maizel (1999) 126: 3183-3190) into the pGEX-4T/C3 vector cut with EcoR I and Sal I. To confirm the sequence of C3APL, the coding sequence from both strands was sequenced. The recombinant C3APL and C3APS cDNAs were separately transformed into bacteria , and after the recombinant proteins were produced, a bacterial homogenate obtained by sonication, and the homogenate cleared by centrifugation. Glutathione-agrose beads (Sigma) were added to the cleared lysate and placed on a rotating plate for 2-3 hours, then washed extensively. To remove the glutathione S transferase sequence from the recombinant protein, 20U of Thrombin was added, the beads were left on a rotator overnight at 4°C. After cleavage with thrombin, the beads were loaded into an empty 20ml column, and the proteins eluted with PBS. Aliquots containing recombinant protein were pooled and 100µls p-aminobenzamidine agrose beads (Sigma) were added and left mixing for 45 minutes at 4°C to remove thrombin, then recombinant protein was isolated from the beads by centrifugation. Purity of the sample was determined by SDS-PAGE, and bioactivity bioassay with PC12 cells. (See Lehmann et al IBID)

Other possible methods for making bioactive chimeric proteins

The Rho antagonist is a recombinant proteins can be made according to methods present in the art. The proteins of the present invention may be prepared from bacterial cell extracts, or through the use of recombinant techniques by transformation, transfection, or infection of a host cell with all or part of a C3-encoding DNA fragment with an antennapedia-derived transport sequence in a suitable expression vehicle. Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems can be used to provide the recombinant protein. The precise host cell used is not critical to the invention.

Any fusion protein can be readily purified by utilising either affinity purification techniques or more traditional column chromatography. Affinity techniques include GST, an antibody specific for the fusion protein being expressed, and histidine-tagged proteins are selectively eluted with imidazole-containing buffers. Alternatively, recombinant protein can be fused to an immunoglobulin Fc domain. Such a fusion protein can be readily purified using a protein A column. It is envisioned that small molecule mimetics of the above described antagonists are also encompassed by the invention.

Testing the bioactivity of C3APL, C3APS, C3TL and C3-TS

To test the efficacy of C3APL , C3APS, C3-TL and C3-TS a number of experiments were performed with PC12 cells, a neural cell line, grown on growth inhibitory substrates. PC12 cells were plated on myelin substrates as described (Lehmann et al , IBID). C3, C3APL , C3APS, C3-TL or C3-TS were added at different concentrations without trituration (please refere to figures 4, 5 and 8for concentrations used). C3 added passively to the culture medium in this way was not able to promote neurite growth in the growth inhibitory substrates because cells must be triturated for C3 to enter the cells and be active (Fig. 1). Both C3APL and C3APLS were able to ADP ribosylates Rho to cause a shift in the molecular weight of RhoA (Figure 2). Both C3APL and C3APLS were able to promote neurite growth and enter neurons after being added passively to the culture medium (Fig. 3, Figure 4 and 5). Dose response experiment where concentrations of 0.25ng/ml, 2.5 ng/ml, 25 ng/ml, 250 ng/ml and 2.5 μg/ml and 25 μg/ml were tested and showed that C3APL and C3APLS helped more neurons differentiate neurites at does 10,000 fold less than C3 (Fig. 4) Dose response experiments where concentrations of 0.25ng/ml, 2.5 ng/ml, 25 ng/ml, 250 ng/ml and 2.5 μg/ml and 25 μg/ml were tested and showed that C3APL was able to promote long neurite growth when added at a minimum concentration of 0.0025 ug/ml (Fig. 5). These concentrations of 2.5 ng/ml and 25 ng/ml for C3APL and C3APLS, represent 10,000 and 1,000 times less than the dose needed with C3, respectively. Moreover, at the highest concentration tested, 50 ug/ml, these two new Rho antagonists did not exhibit toxic effect on PC12 cells, and were able to stimulate neurite outgrowth on growth inhibitory substrates.

C3-TL and C3-TS also were tested at concentrations of 0.25ng/ml, 2.5 ng/ml, 25 ng/ml, 250 ng/ml and 2.5 μg/ml and 25 μg/ml and were found to be able to promote neurite growth on myelin substrates at doses significantly less than C3 (Figure 8).

To verify the ability of C3APL and C3APS to promote growth from primary neurons, primary retinal cultures were prepared, and the neurons were plated on myelin substrates as described with respect to example 5. In the absence of treatment with C3APL orC3APS, the cells remained round and are not able to grow neurites. When treated with C3APL or C3APS, retinal neurons were able to extend long neurites on inhibitory myelin substrates (Figure 6).

Next, was tested the ability of C3APL and C3APS to promote growth on a different type of growth inhibitory substrate relevant to the type of growth inhibitory proteins found at glial scars. Chamber slides were coated with a mixture of chondroitin sulfate proteoglycans (Chemicon), and then plated retinal neurons. The neurons were not able to extend neuritis on the proteoglycan substrates, but when treated with C3APL or C3APS, they extended long neurites (not shown). These studies demonstrate that C3APL and C3APS can be used to promote neurite growth on myelin and on proteoglycans, the major classes of inhibitory substrates that prevent repair after injury in the CNS.

Testing ability of C3APL to promote regeneration and functional recovery after spinal cord injury

To test if C3APL could promote repair after spinal cord injury, fully adult mice were used.(as described with resepcet to example 6). A dorsal hemisection was made at T8, and mice were treated with different amounts (figure 7) of C3APL in a fibrin glue as described (McKerracher, US patent pending (delivery patent)). In previous known experiments with C3, it was found that 40-50 ug was needed to promote anatomical regeneration in optic nerve (Lehmann et all IBID). We tested different doses (see figure 7) of C3APL ranging form 1 ug to 50 ug and assessed animals for behavioural recovery according the BBB scale (Basso (1995) 12: 1-21)

The day following surgery and application of C3APL behavioural testing began The animals were placed in an open field environment that consisted of a rubber mat approximately 4' X

3' in size. The animals were left to move randomly, the movement of the animals were videotaped. For each test two observers scored the animals for ability to move ankle, knee and hip joints in the early phase of recovery. Previously C3 treatment of mice was seen to lead to functional recovery observable 24 hours after treatment. In mice treated with C3APL, functional recovery could be observed as early as 24 hours after spinal cord injury (Fig. 7). Untreated mice exhibit a function recovery score according to the BBB scale averaging 0, whereas mice treated with C3 are able to walk and have a BBB score averaging 8 (Fig.7). At higher concentrations of 50 ug the mice died within 24 hours. However, of the mice that survive, they exhibited good long-term functional recovery. These results demonstrate that C3APL effectively promotes functional recovery early after spinal cord injury, and that it is effective at much lower doses than C3. However, at high concentrations, C3APL appears to exhibit toxicity, and therefore careful doing will be required for clinical use.

Qualitative observations of the video tapes showed thatoOnly animals that received C3APL reached the late phase of recovery after 30 days of treatment. Untreated control animals did not typically pass beyond the early phase of recovery. These results indicate that the application of C3APL improved long-term functional recovery after spinal cord injury compared to no treatement, injury alone, or fibrin adhesive alone.

EXAMPLE 1. DNA and protein sequence details of C3APL

Nucleotide sequence of C3APL. The start site, is in the GST sequence of the plasmid (not shown). The vector with the GST sequence is commercially available and thus the entire GST sequence including the start was not sequenced. It was desired to determine only the sequence 3 ' to the thrombin cleavage site which releases C3 conjugate from the GST sequence. The GST sequence is cleaved with thrombin.

Both strands were sequenced to verify that there were no errors in the sequence.

Bold is the stop codon.

Nucleotide sequence of coding sequence for the protein C3APL (SEQ ID NO: 3)

5' GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT
TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA
TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA
AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
GCT ATC AAT CCT AAA GAA TTC GTG ATG GAA TCC CGC AAA CGC GCA AGG
CAG ACA TAC ACC CGG TAC CAG ACT CTA GAG CTA GAG AAG GAG TTT CAC
TTC AAT CGC TAC TTG ACC CGT CGG CGA AGG ATC GAG ATC GCC CAC GCC
CTG TGC CTC ACG GAG CGC CAG ATA AAG ATT TGG TTC CAG AAT CGG CGC
ATG AAG TGG AAG AAG GAG AAC TGA 3'

Amino acid sequence of C3APL (SEQ ID NO: 4)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
YTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPENIMLFRGDDPAYLG
TEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITQFKVA

KGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFV
MESRKRARQTYTRYQTLELEKEFHFNRYLTRRRRIEIAHALCLTERQIKIWFQNRRMK
WKKEN

Physical characteristics of C3APL

Molecular Weight 34098.03 Daltons

295 Amino Acids

48 Strongly Basic(+) Amino Acids (K,R)

28 Strongly Acidic(-) Amino Acids (D,E)

89 Hydrophobic Amino Acids (A,I,L,F,W,V)

94 Polar Amino Acids (N,C,Q,S,T,Y)

9.847 Isolectric Point

20.524 Charge at PH 7.0

Davis,Botstein,Roth Melting Temp C. 79.48

EXAMPLE 2. DNA and protein sequence details of C3APSt

Nucleotide sequence of C3APS (SEQ ID NO: 5). The start site, is in the GST sequence of the plasmid, not shown here.

5' GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG

AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT
TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA
TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA
AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
GCT ATC AAT CCT AAA GAA TTC CGC CAG ATC AAG ATT TGG TTC CAG AAT
CGT CGC ATG AAG TGG AAG AAG GTC GAC TCG AGC GGC CGC ATC GTG ACT
GAC TGA 3'

Amino acid sequence for C3APS (SEQ ID NO: 6)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
YTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPENIMLFRGDDPAYLG
TEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITQFKVA
KGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFR
QIKIWFQNRRMKWKKVDSSGRIVTD

Physical charteristics of C3APS

Molecular Weight 29088.22 Daltons

257 Amino Acids

38 Strongly Basic(+) Amino Acids (K,R)

23 Strongly Acidic(-) Amino Acids (D,E)

79 Hydrophobic Amino Acids (A,I,L,F,W,V)

83 Polar Amino Acids (N,C,Q,S,T,Y)

9.745 Isolectric Point

15.211 Charge at PH 7.0

Davis,Botstein,Roth Melting Temp C. 78.34

EXAMPLE 3. METHOD FOR MAKING THE C3APL AND C3APS PROTEINS

C3APL is the name given to the protein encoded by cDNA made by ligating the functional domain of C3 transferase and the homeobox region of the transcription factor called anntenopedia (Bloch-Gallego (1993) 120: 485-492) in the following way. A cDNA encoding C3 (Dillon and Feig (1995) 256: 174-184) the plasmid vector pGEX-2T was used for the C3 portion of the chimeric protein. The stop codon at the 3' end of the DNA was replaced with an EcoR1 site by polymerase chain reaction using the primers 5'GAA TTC TTT AGG ATT GAT AGC TGT GCC 3' (SEQ ID NO: 1) and 5'GGT GGC GAC CAT CCT CCA AAA 3' (SEQ ID NO: 2). The PCR product was sub-cloned into a pSTBlue-1 vector (Novagen, city), then cloned into a pGEX-4T vector using BamH I and Not I restriction site. This vector was called pGEX-4T/C3. The pGEX-4T vector has a 5' glutathione S transferase (GST), sequence for use in affinity purification. The anntenopedia sequence used to add to the 3' end of C3 in pGEX-4T/C3 was created by PCR from the pET-3a vector (Bloch-Gallego (1993) 120: 485-492, Derossi (1994) 269: 10444-10450). The primers used were 5'GAA TCC CGC AAA CGC GCA AGG CAG 3' (SEQ ID NO: 7) and 5'TCA GTT CTC CTT CTT CCA CTT CAT GCG 3' (SEQ ID NO: 8). The PCR product obtained from the reaction was subcloned into a pSTBlue-1 blunt vector, then cloned into the pGEX-4T/C3, using the restriction sites EcoR I and Sal I, creating pGEX-4T/C3APL.

A shorter version of the Antennapedia (pGEX-4T/C3AP-short) was also made. This chimeric sequence was made by ligating oligonucleotides encoding the short anntenapedia pepide (Maizel (1999) 126: 3183-3190) into the pGEX-4T/C3 vector cut with EcoR I and Sal I. For pGEX-4T/C3AP-short the sequences of the oligos made were 5'AAT TCC GCC AGA TCA AGA TTT GGT TCC AGA ATC GTC GCA TGA AGT GGA AGA AGG 3' (SEQ ID NO: 9)

and 5'GGC GGT CTA GTT CTA AAC CAA GCT CTT AGC AGC GTA GTT CAC CTT CTT CCA GCT 3' (SEQ ID NO: 10). The two strands were annealed together by mixing equal amounts of the oligonucleotides, heating at 72 °C for 5 minutes and then leaving them at room temperature for 15 minutes. The oligonucleotides were ligated into the pGEX4T/C3 vector. To confirm the sequences of C3APL, the coding sequence from both strands of pGEX-4T/C3APL were sequenced.

To prepare recombinant C3APL and C3APS proteins, the plasmids containing the corresponding cDNAs (pGEX-4T/C3APL and pGEX-4T/C3AP-short) were transformed into bacteria , strain XL-1 blue competent E. coli. The bacteria were grow were grown in L-broth (10g/L Bacto-Tryptone, 5g/L Yeast Extract, 10g/L NaCl) with ampicillin at 50 ug/ml(BMC-Roche), in a shaking incubator for 1 hr at 37 °C and 300 rpm. Isopropyl β-thiogalactopyranoside (IPTG), (Gibco) was added to a final concentration of 0.5 mM to induce the production of recombinant protein and the culture was grown for a further 6 hours at 37° C and 250 rpm. Bacteria pellets were obtained by centrifugation in 250 ml centrifuge bottles at 7000rpm for 6 minutes at 4 °C. Each pellet was re-suspended in 10 ml of Buffer A (50mM Tris, pH 7.5, 50mM NaCl, 5mM $MgCl_2$, 1mM DTT) plus 1mM PMSF. All re-suspended pellets were pooled and transferred to a 100 ml plastic beaker on ice. The remaining buffer A with PMSF was added to the pooled sample. The bacteria sample was sonicated 6 x 20 seconds using a Branson Sonifier 450 probe sonicator. Both the bacteria and probe were cooled on ice 1 minute between sonications. The sonicate was centrifuged in a Sorvall SS-34 rotor at 16,000 rpm for 12 minutes at 4°C to clarify the supernatant. The supernatant was transferred into fresh SS-34 tubes and re-spun at 12,000 rpm for 12 minutes at 4°C. Up to 20 ml of Glutathione-agrose beads (Sigma) were added to the cleared lysate and placed on a rotating plate for 2-3 hours. The beads were washed 4 times with buffer B, (buffer A, NaCl is 150mM, no PSMF) then 2 times with buffer C (buffer B + 2.5mM $CaCl_2$). The final wash was poured out till the beads created a thick slurry. To remove the glutathione S transferase sequence from the recombinant protein, 20U of Thrombin (Bovine, Plasminogen-free, Calbiochem) was added, the beads were left on a rotator overnight at 4°C. After cleavage with thrombin the beads were loaded into an empty 20ml column. Approximately 20 aliquots of 1 ml were collected by elution with PBS. Samples of each aliquote of 0.5ul were spotted on nitrocellulose and stained with Amido Black to determine

the protein peak. Aliquots containing C3 were pooled and 100µls p-aminobenzamidine agrose beads (Sigma) were added and left mixing for 45 minutes at 4°C. This last step removed the thrombin from the recombinant protein sample. The recombinant protein was centrifuged to remove the beads and then concentrated using a centriprep-10 concentrator (Amicon). The concentrated recombinant protein was desalted with a PD-10 column (Pharmacia, containing Sephadex G-25M) and 10 0.5ml aliquots were collected. A dot-blot was done on these samples to determine the protein peak, and the appropriate aliquots pooled, filter-sterilized, and stored at – 80°C. A protein assay (Biorad) was used to determine the concentration of recombinant protein. Purity of the sample was determined by SDS-PAGE, and bioactivity bioassay with PC12 cells.

EXAMPLE 4: TESTING OF EFFECACY OF C3APL AND C3APS IN TISSUE CULTURE

To test the ability of C3APL and C3APS to overcome growth inhibition, PC12 cells were plated on myelin, a growth inhibitory substrate. The myelin was purified from bovine brain (Norton and Poduslo (1973) 21: 749-757). In some other experiments chondroitin sulfate proteoglycan (CSPG) substrates were made from a purchased protein composition (Chemicon). Before coating coverslips or wells of a 96 well plate, they were coated with poly-L-lysine (0.025µg/ml) (Sigma, St. Louis, MO, washed with water and allowed to dry. Myelin stored as a 1mg/ml solution at –80 C was thawed at 37C, and vortexed. The myelin was plated at 8 ug/well of a 8 well chamber Lab-Tek slides (Nuc, Naperville, IL). The myelin solution was left to dry overnight in a sterile tissue culture hood. The next morning the substrate was washed gently with phosphate buffered saline, and then cells in media were added to the substrate. PC-12 cells (Lehmann et al., 1999) were grown in DMEM with 10% horse serum (HS) and 5% fetal bovine serum (FBS). Two days prior to use the PC-12 cells were differentiated by 50 ng/ml of nerve growth factor (NGF). After the cells were primed, 5ml of trypsin was added to the culture dish detach the cells, the cells were pelleted and re-suspended in 2ml of DMEM with 1% HS and 50 ng/ml of nerve growth factor. Approximately, 5000 to 7000 cells were then plated on 8 well chamber Lab-Tek slides (Nuc, Naperville, IL) coated myelin. The cells were placed on the test substrates at 37°C for 3-4 hours to allow the cells to settle. The original media was carefully removed by aspiration,

taking care not to disrupt the cells and replaced with DMEM with 1% HS, 50ng/ml of NGF and varying amounts of the C3, C3APL, or C3APS, depending on the dose desired. After two days, the cells were fixed (4% paraformaldehyde and 0.5% glutaraldehyde). For control experiments with unmodified C3, NGF primed PC12 cells were trypsinized to detach them form the culture dish, the cells were washed once with scrape loading buffer (in mM: 114 KCL, 15 NaCl, 5.5 $MgCl_2$, and 10 Tris-HCL) and then the cells were scraped with a rubber policeman into 0.5 ml of scraping buffer in the presence of 25 or 50 μg/ml C3 transferase. The cells were pelleted and resuspended in 2ml of DMEM, 1% HS and 50 ng/ml nerve growth factor before plating. At least four experiments were analyzed for each treatment. For each well, twelve images were collected with a 20X objective using a Zeiss Axiovert microscope. For each image, the numbers of cells with and without neurites were counted and the lengths of the neurites were determined. Since myelin is phase dense, cells plated on myelin substrates were immuno-stained with anti-βIII tubulin antibody before analysis. Quantitative analysis of neurite outgrowth was with the aid of Northern Eclipse software (Empix Imaging, Mississauga, Ontario, Canada). Data analysis and statistics were with Microsoft Excel.

To examine ADP ribosylation by C3, C3APL, and C3APS, the compounds were added to PC12 cell cultures, as described above. The cells were harvested by centrifugation, cell homogenates prepared and the proteins separated by SDS polyacrylamidegel electrophoresis. The Proteins were then transfered to nitrocellulose and the Western blots probed with anti-Rho Antibody (UBI).

EXAMPLE 5: TESTING ABILITY OF C3APL AND C3APS TO OVERRIDE INHIBITION OF MULTIPLE GROWTH INHIBITORY PROTEINS

Myelin substrates were made as described in example 4 and plated on tissue culture chamber slides. P1 to P3 rat pups were decapitated, the heads washed in ethanol and the eye removed and placed in a petri dish with Hanks buffered saline solution (HBSS, from Gibco). A hoel was cut in the cornea, the lens removed, and the retina squeezed out. Typically, four retinas

per preparation were used. The retinas were removed to a 15 ml tube and the volume brought to 7 ml. A further 7 mls of dissociation enzymes and papain were added. The dissociation enzyme solution was made as follows: 30 mg DL cystein was added to a 15 ml tube (Sigma DK cyctein hydrochloride), and 70 ml HBSS, 280 ul of 10mg.ml bovine serum albumin were added and the solution mixed and pH adjusted to 7 with 0.3 N NaOH. The dissociate solution was filter-sterilized and kept frozen in 7 ml aliquots, and before use 12.5 units papain per ml (Worthington) was added. After adding the dissociation solution to the retina, the tube was incubated for 30 min. on a rocking tray at 37C. The retinas were then gently triturated, centrifuged and washed with HBSS. The HBSS was replaced with growth medium (DMEM (Gibco), 10 % fetal bovine serum, and 50 ng/ml brain derived neurotrophic factor (BDNF) vitamins, penicillin-streptomycin, in the presence or absence of C3APL or C3APS. Cells were plated on test substrates of myelin or CSPG in chamber slides prepared as described in example 4 , above. A quantitative analysis was completed as described for example 4 above. Neurons were visualized by fluorescent microscopy with anti-βIII tublin antibody, which detects growing retinal ganglion cells (RGCs).

EXAMPLE 6. TREATMENT OF INJURED MOUSE SPINAL CORD WITH C3APL AND MEASUREMENT OF RECOVERY OF MOTOR FUNCTION IN TREATED MICE.

Adult Balb-c mice were anaesthetized with 0.6 ml/kg hypnorm, 2.5 mg/kg diazepam and 35 mg/kg ketamine. This does gives about 30 minutes of anesthetic, which is sufficient for the entire operation. A segment of the thorac spinal column was exposed by removing the vertebrae and spinus processus with microrongeurs (Fine Science Tools). A spinal cord lesion was then made dorsally, extending past the central canal with fine scissors, and the lesion was recut with a fine knife. This lesion renders all of the control animals parapelegic. The paravertebral muscle were closed with reabsorbable sutures, and the skin was closed with 2.0 silk sutures. After surgery, the

bladder was manually voided every 8-10 hours until the animals regained control, typically 2-3 days. Food was placed in the cage for easy access, and sponge-water used for easy accessibility of water after surgery. Also, animals received subcutaneous injection Buprenorphine (0.05 à 0.1 mg/kg) every 8-12 hours for the first 3 days. Any animals that lost 15-20% of body weight were killed.

Rho antagonists were delivered locally to the site of the lesion by a fibrin-based tissue adhesive delivery system (McKerracher, US patent submitted). Recombinant C3APL was mixed with fibrinogen and thrombin in the presence of $CaCl_2$. Fibrinogen is cleaved by thrombin, and the resulting fibrin monomers polymerize into a three-dimensional matrix. We added C3APL as part of a fibrin adhesive, which polymerized within about 10 seconds after being placed in the injured spinal cord. We tested C3APL applied to the spinal cord lesion site after the lesion was made. For control we injected fibrin adhesive alone, or transected the cord without further treatment. For behavioural testing, the BBB scoring method was used to examine locomotion in an opern field environment (Basso (1995) 12: 1-21). The environment was a rubber mat mat approximately 4' X 3' in size, and animals were placed on the mat and videotaped for about 4 minutes. Care was taken not to stimulate the peroneal region or touch the animals excessively during the taping session. The video tapes were digitized and observed by two observers to assign BBB scores. The BBB score, modified for mice, was as follows:

Score	**Description**
1	No observable hindlimb (HL) movement.
2	Slight movement of one or two joints.
3	Extensive movement of one joint and /or slight movement of one other joint..
4	Extensive movement of two joints.
5	Slight movement of all three joints of the HL.
6	Slight movement of two joints and extensive movement of the third.
7	Extensive movement of two joints and slight movement of the third.
8	Extensive movement of all three joints of the HL walking with no weight support.
9	Extensive movement of all three joints, walking with weight support.
10	Frequent to consistent dorsal stepping with weight support.

11 Frequent plantar stepping with weight support.

12 Consistent plantar stepping with weight support, no coordination.

13 Consistent plantar stepping with consistent weight support, occasional FL-HL coordination.

14 Consistent plantar stepping with consistent weight support, frequent FL-HL coordination.

15 Consistent plantar stepping with consistent weight support, consistent FL-HL coordination; Predominant paw position during locomotion is rotated internally or externally, or consistent FL-HL coordination with occasional dorsal stepping.

16 Consistent plantar stepping with consistent weight support, consistent FL-HL coordination; predominant paw position is parallel to the body; frequent to consistent toe drag, or curled toes, trunk instability.

17 Consistent plantar stepping with consistent weight support, consistent FL-HL coordination; predominant paw position is parallel to the body, no toe drag, some trunk instability.

18 Consistent plantar stepping with consistent weight support, consistent FL-HL coordination; predominant paw position is parallel to the body, no toe drag and consistent stability in the locomotion.

EXAMPLE . 7 TREATMENT OF INJURED MOUSE SPINAL CORD WITH C3APL AND ASSESSMENT OF ANATOMICAL RECOVERY.

Mice that received a spinal cord injury and treated as controls or with C3APL, as described for example 6 were assessed for morphological changes to the scar and for axon regeneration. To study axon regeneration, the corticospinal axons were identified by anterograde labeling. For anterograde labeling studies, the animals were anaesthetized as above, and the cranium over the motor cortex was removed. With the fine glass micropippetter (about 100 um in diameter) le cerbral cortex was injected with 2-4 ul of horse radishe peroxidase conjugated to wheat germ agglutinin (2%), a marquer that is taken up by nerve cells and transported antergradely into the axon that extends into the spinal cord. After injection of the anterograde tracer, the cranium was replaced, and the skin closed with 5-0 silk sutures. The animals were

sacrificed with chloral hydrate (4.9 mg/10 g) after 48 hours, and perfused with 4% paraformaldehyde in phosphate buffer as a fixative. The spinal cord was removed, cryoprotected with sucrose and cryostat sections placed on slides for histological examination.

EXAMPLE 8. DNA and protein sequence details of C3-TL

The TAT coding sequence was obtained by polymerase chain reaction of the plasmid SVCMV-TAT(obtained form Dr. Eric Cohen, Universite de Montreal) that contains the entire Tat coding sequence. To isolate the transport sequence of the Tat protein PCR was used. The first primer (5'GAATCCAAGCACCAGGAAGTCAGCC 3'- (SEQ ID NO: 11)) and the second primer (5' ACC AGCCACCACCTTCTGATA 3' - (seq id no: 12)) used corresponded to amino acids 27 to 72 of the HIV TAT protein. Upon verification and purification, the PCR product was sub cloned into a pSTBlue-1 blunt vector. This transport segment of the TAT protein was then cloned into pGEX-4T/C3, using the restriction sites EcoR I and Sac I. The new C3-tat fusion protein was called C3-TL. Recombinant protein was made as described in Example 3.

DNA sequence of C3-TL (seq id no: 13)

5' GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT
TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA
TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA
AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
GCT ATC AAT CCT AAA GAA TTC AAG CAT CCA GGA AGT CAG CCT AAA ACT

GCT TGT ACC AAT TGC TAT TGT AAA AAG TGT TGC TTT CAT TGC CAA GTT TGT TTC ATA ACA AAA GCC TTA GGC ATC TCC TAT GGC AGG AAG CGG AGA CAG CGA CGA AGA GCT CAT CAG AAC AGT CAG ACT CAT CAA GCT TCT CTA TCA AAG CAG TAA 3'

The protein sequence of C3-TL (seq id no: 14)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS YTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPENIMLFRGDDPAYLG TEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITQFKVA KGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFK HPGSQPKTACTNCYCKKCCFHCQVCFITKALGISYGRKRRQRRRAHQNSQTHQASLS KQ.

Molecular Weight 32721.40 Daltons

291 Amino Acids

43 Strongly Basic(+) Amino Acids (K,R)

21 Strongly Acidic(-) Amino Acids (D,E)

82 Hydrophobic Amino Acids (A,I,L,F,W,V)

104 Polar Amino Acids (N,C,Q,S,T,Y)

9.688 Isolectric Point

22.655 Charge at PH 7.0

Total number of bases translated is 876

% A = 37.44 [328]

% G = 17.58 [154]

% T = 28.31 [248]

% C = 16.67 [146]

EXAMPLE 9. DNA AND PROTEIN SEQUENCE DETAILS OF C3-TS

A shorter tat construct was also made called C3-TS. To make the shorter C3 tat fusinon protein the following oligonucleotrides were 5'AAT TCT ATG GTC GTA AAA AAC GTC GTC AAC GTC GTC GTG 3' (SEQ ID NO: 15)

and 5' GAT ACC AGC ATT TTT TGC AGC AGT TGC AGC AGC ACA GCT 3' (SEQ ID NO: 16. The two oligonucleotide strands were annealed together by combining equal amounts of the oligonucleotides, heating at 72 °C for 5 minutes and then letting the oligonucleotide solution cool at room temperature for 15 minutes. . The oligonucleotides were ligated into the pGEX4T/C3 vector. The constructs was sequenced. All plasmids were transformed into XL-1 blue competent cells. Recombinant protein was made as described in Example 3.

Nucleotide sequence of C3-TS (SEQ ID NO: 17)

5' GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT

AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT AAA GAA TTC TAT GGT GCT AAA AAA CGT CGT CAA CGT CGT CGT GTC GAC TCG AGC GGC CCG CAT CGT GAC TGA 3'

The protein sequence of C3-TS (SEQ ID NO: 18)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS YTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPENIMLFRGDDPAYLG TEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITQFKVA KGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFY GAKKRRQRRRVDSSGPHRD

Molecular Weight 26866.62 Daltons
238 Amino Acids
36 Strongly Basic(+) Amino Acids (K,R)
21 Strongly Acidic(-) Amino Acids (D,E)
71 Hydrophobic Amino Acids (A,I,L,F,W,V)
78 Polar Amino Acids (N,C,Q,S,T,Y)

9.802 Isolectric Point
15.212 Charge at PH 7.0

Total number of bases translated is 717
% A = 38.91 [279]
% G = 17.43 [125]

% T = 28.45 [204]
% C = 15.20 [109]

EXAMPLE 10.

The following example illustrates how a coding sequence can be modified without affecting the efficacy of the transplated protein. The example shows modifications to C3basic3 that would not affect the activity. Sequences may include the entire GST sequence, as shown here that includes the start site, which would not be removed enzymatically. Also, the transport sequence shown in this example has changes in amino acid composition surrounding the active sequence dues to a difference in the cloning strategy, and the his tag has been omitted. However, the active region is : R R K Q R R K R R . This sequence is contained in the C3BASIC3, and is the active transport sequence in the sequence below. Also note that the C-terminal region of the protein after this active region differs from C3BASIC1. That is because the cloning strategy was changed, the restriction sites differ, and therefore non-essential amino acids 3 terminal to the transport sequence are transplated and included in the protein.

Nucleic acid sequence: (SEQ ID NO: 19)
1413 base pairs
single strand
liniear sequence

5' ATG TCC CCT ATA CTA GGT TAT TGG AAA ATT AAG GGC CTT GTG CAA CCC ACT CGA CTT CTT TTG GAA TAT CTT GAA GAA AAA TAT GAA GAG CAT TTG TAT GAG CGC GAT GAA GGT GAT AAA TGG CGA AAC AAA AAG TTT GAA TTG GGT TTG GAG TTT CCC AAT CTT CCT TAT TAT ATT GAT GGT GAT GTT AAA TTA ACA CAG TCT ATG GCC ATC ATA CGT TAT ATA GCT GAC AAG CAC AAC ATG TTG GGT GGT TGT CCA AAA GAG CGT GCA GAG ATT TCA ATG CTT GAA GGA GCG GTT TTG GAT ATT AGA TAC GGT GTT TCG AGA ATT GCA TAT AGT AAA GAC TTT GAA ACT CTC AAA GTT GAT TTT CTT AGC AAG CTA CCT GAA

ATG CTG AAA ATG TTC GAA GAT CGT TTA TGT CAT AAA ACA TAT TTA AAT
GGT GAT CAT GTA ACC CAT CCT GAC TTC ATG TTG TAT GAC GCT CTT GAT
GTT GTT TTA TAC ATG GAC CCA ATG TGC CTG GAT GCG TTC CCA AAA TTA
GTT TGT TTT AAA AAA CGT ATT GAA GCT ATC CCA CAA ATT GAT AAG TAC
TTG AAA TCC AGC AAG TAT ATA GCA TGG CCT TTG CAG GGC TGG CAA GCC
ACG TTT GGT GGT GGC GAC CAT CCT CCA AAA TCG GAT CTG GTT CCG CGT
GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
AAG ACC CCT GAA AAT ATT ATG TTA TTT ANA GGC GAC GAC CCT GCT TAT
TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT ANA GAT
AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA
TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA
AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
GCT ATC AAT CCT AAA GAA TTC AGA AGG AAA CAA AGA AGA A AA AGA
AGA CTG CAG GCG GCC GCA TCG TGA 3'

Amino acid sequence (SEQ ID NO: 20)

479 amino acids

linear, single strand

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPY
YIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDF
ETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALDVVLYMDPMCL
DAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLVPRGSSR

VDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVSYTKS
ASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPENIMLFXGDDPAYLGTEFQ
NTLLNSNGTINKTAFEKAKAKFLNXDRLEYGYISTSLMNVSQFAGRPIITKFKVAKGS
KAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFRRKQ
RRKRRLQAAAS.

Molecular Weight 53813.02 Daltons

470 Amino Acids

68 Strongly Basic(+) Amino Acids (K,R)

55 Strongly Acidic(-) Amino Acids (D,E)

149 Hydrophobic Amino Acids (A,I,L,F,W,V)

121 Polar Amino Acids (N,C,Q,S,T,Y)

9.137 Isolectric Point

14.106 Charge at PH 7.0

Total number of bases translated is 1413

% A = 34.61 [489]

% G = 19.75 [279]

% T = 29.51 [417]

% C = 15.99 [226]

% Ambiguous = 0.14 [2]

% A+T = 64.12 [906]

% C+G = 35.74 [505]

Davis,Botstein,Roth Melting Temp C. 79.20

EXAMPLE 11. ADDITIONAL CHIMERIC C3 PROTEINS THAT WOULD BE EFFECTIVE TO STIMULATE REPAIR IN THE CNS.

The following sequences could be added to the amino terminal or carboxy terminal of C3 or a truncated C3 that retains its enzymatic activity.

1) Sequences of polyarginine as decribed (Wender, et al. (2000) 97: 13003-8.). These could be from 6 to 9 or more arginines.
2) Sequences of poly-lSyine
3) Sequences of polyhistidine
4) Sequences of arginine and lysine mixed.
5) Basic stretchs of amino acids containing non-basic amino acids stretch where the sequence added retains transport characteristics.
6) Sequences of 5- 15 amino acids containing at least 50 % basic amino acids
7) Sequences longer than 15 –30 amino acids containing at least 30 % basic amino acids.
8) Sequences longer than 50 amino acids containing at least 18 % basic amino acids.
9) Any of the above where the amino acids are chemically modified, such as by addition of cyclohexl side chains, other side chains, different alkyl spacers.

EXAMPLE 12. ADDITIONAL CHIMERIC C3 PROTEINS THAT WOULD BE EFFECTIVE TO STIMULATE REPAIR IN THE CNS.

ENBbu

C3basic1: C3 fused to a randomly designed basic tail

C3basic2: C3 fused to a randomly designed basic tail

C3 basic3: C3 fused to the reverse Tat sequence

EXAMPLE 12. ADDITIONAL CHIMERIC C3 PROTEIN THAT WOULD BE EFFECTIVE TO STIMULATE REPAIR IN THE CNS.

We have designed the following DNA encoding a chimeric C3 with membrane transport properties. The protein is designated **C3BASIC1**. This sequence was designed with C3 fused to a random basic sequence. The construct was made to encode the peptide given below.

K R R R R R P K K R R R A K R R (SEQ ID NO: 21)

The construct was made by synthesizing the two oligonucleotides given below, annealing them together, and ligating them into the pGEX-4T/C3 vector with an added histidine tag.

5' AAG AGA AGG CGA AGA AGA CCT AAG AAG AGA CGA AGG GCG AAG AGG AGA 3' (SEQ ID NO: 22)

5' TTC TCT TCC GCT TCT TCT GGA TTC TTC TCT GCT TCC CGC TTC TCC TCT 3' (SEQ ID NO 23)

DNA sequence C3BASIC1 (SEQ ID NO: 24)

5' GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA AAT ATT ATG TTA TTT ANA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT ANA GAT AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT AAA GAA TTC AAG AGA AGG CGA AGA AGA CCT AAG AAG AGA CGA AGG GCG AAG AGG AGA CAC CAC CAC CAC CAC CAC GTC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA 3'

Protein sequence (SEQ ID NO: 25)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
YTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPENIMLFXGDDPAYLG
TEFQNTLLNSNGTINKTAFEKAKAKFLNXDRLEYGYISTSLMNVSQFAGRPIITKFKVA
KGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFK
RRRRRPKKRRRAKRRHHHHHHVDSSGRIVTD.

Molecular Weight 29897.03 Daltons

263 Amino Acids

44 Strongly Basic(+) Amino Acids (K,R)

23 Strongly Acidic(-) Amino Acids (D,E)

75 Hydrophobic Amino Acids (A,I,L,F,W,V)

79 Polar Amino Acids (N,C,Q,S,T,Y)

10.024 Isolectric Point

22.209 Charge at PH 7.0

Davis,Botstein,Roth Melting Temp C. 78.56

EXAMPLE 13. ADDITIONAL CHIMERIC C3 PROTEIN THAT WOULD BE EFFECTIVE TO STIMULATE REPAIR IN THE CNS.

We have designed the following DNA encoding a chimeric C3 with membrane transport properties. The protein is designated **C3BASIC2**. This sequence was designed with C3 fused to a random basic sequence. The construct was made to encode the peptide given below.

K R R R R K K R R Q R R R (SEQ ID NO: 26)

The construct was made by synthesizing the two oligonucleotides given below, annealing them together, and ligating them into the pGEX4T/C3 vector with an added histidine tag.

5' AAG CGT CGA CGT AGA AAG AAA CGT AGA CAG CGT AGA CGT 3' (SEQ ID NO: 27)

5' TTC GCA GCT GCA TCT TTC TTT GCA TCT GTC GCA TCT GCA 3' (SEQ ID NO: 28)

DNA sequence C3BASIC2 (SEQ ID NO: 29)

5' GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA AAT ATT ATG TTA TTT ANA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT ANA GAT AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT AAA GAA TTC AAG CGT CGA CGT AGA AAG AAA CGT AGA CAG CGT AGA CGT CAC CAC CAC CAC CAC CAC GTC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA 3'

Protein sequence (SEQ ID NO: 30)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS YTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPENIMLFXGDDPAYLG TEFQNTLLNSNGTINKTAFEKAKAKFLNXDRLEYGYISTSLMNVSQFAGRPIITKFKVA KGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFK RRRRKKRRQRRRHHHHHHVDSSGRIVTD.

Molecular Weight 29572.61 Daltons

260 Amino Acids

42 Strongly Basic(+) Amino Acids (K,R)

23 Strongly Acidic(-) Amino Acids (D,E)

74 Hydrophobic Amino Acids (A,I,L,F,W,V)

80 Polar Amino Acids (N,C,Q,S,T,Y)

9.956 Isolectric Point

20.210 Charge at PH 7.0

Davis,Botstein,Roth Melting Temp C. 78.45

EXAMPLE 14. ADDITIONAL CHIMERIC C3 PROTEIN THAT WOULD BE EFFECTIVE TO STIMULATE REPAIR IN THE CNS.

We have designed the following DNA encoding a chimeric C3 with membrane transport properties. The protein is designated **C3BASIC3**. This sequence was designed with C3 fused to a the reverse Tat sequence. The construct was made to encode the peptide given below

R R K Q R R K R R (SEQ ID NO: 31)

The construct was made by synthesizing the two oligonucleotides given below, annealing them together, and ligating them into the pGEX4T/C3 vector with an added histidine tag, then subcloning inot pGEX-4T/C3.

5' AGA AGG AAA CAA AGA AGA AAA AGA AGA 3' (SEQ ID NO: 32)

5' TCT TCC TTT GTT TCT TCT TTT TCT TCT 3' (SEQ ID NO: 33)

DNA sequence **C3BASIC3** (SEQ ID NO: 34)

5' GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA

GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
AAG ACC CCT GAA AAT ATT ATG TTA TTT ANA GGC GAC GAC CCT GCT TAT
TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT ANA GAT
AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA
TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA
AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
GCT ATC AAT CCT AAA GAA TTC AGA AGG AAA CAA AGA AGA AAA AGA
AGA CAC CAC CAC CAC CAC CAC GTC GAC TCG AGC GGC CGC ATC GTG ACT
GAC TGA 3'

Protein sequence (SEQ ID NO: 35)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
YTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPENIMLFXGDDPAYLG
TEFQNTLLNSNGTINKTAFEKAKAKFLNXDRLEYGYISTSLMNVSQFAGRPIITKFKVA
KGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFR
RKQRRKRRHHHHHHVDSSGRIVTD.

Molecular Weight 29441.47 Daltons

260 Amino Acids

39 Strongly Basic(+) Amino Acids (K,R)

23 Stron.gly Acidic(-) Amino Acids (D,E)

76 Hydrophobic Amino Acids (A,I,L,F,W,V)

80 Polar Amino Acids (N,C,Q,S,T,Y)

9.833 Isolectric Point

17.211 Charge at PH 7.0

Davis,Botstein,Roth Melting Temp C. 78.29

Example 15: Modifications of sequences. Any of sequences given in examples 1, 2, 8, 9, 10, 11, 12 and 13 could be modified to retain C3 enzymatic activity(and effective transport

sequences). For example amino acids encoded from DNA at the 3' end of the sequence that represents the translation of the restriction sites used in cloning can be removed without affecting activity. Some of the amino terminal amino acids may also be removed without affecting activity. The C3 portion of teh protein could be truncated to include just the amino acids needed for activity. The transport sequences could be modified to add or remove one or more amino unessential acids.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: LISA MCKERRACHER

(ii) TITLE OF INVENTION: FUSION PROTEINS

(iii) NUMBER OF SEQUENCES: 35

(iv) CORRESPONDENCE ADDRESS:
(A) ADRESSEE: BROULLETTE KOSIE
(B) STREET: 1100 RENE-LESVEQUE BLVD WEST
(C) PROV/STATE: QUEBEC
(D) COUNTRY: CANADA
(E) POSTAL/ZIP CODE: H3B 5C9

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE:ASCII (TEXT)

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) ATTORNEY/AGENT INFORMATION:
(A) NAME: BROULLETTE KOSIE

(B) REGISTRATION NO.:

(C) REFERENCE/DOCKET NO.: 06447-004-CA-01

(D) TEL. NO.: (514) 397 8500

(E) FAX NO.: (514) 397 8515

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE: 5' cDNA primer - C3 sequence

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

5'GAA TTC TTT AGG ATT GAT AGC TGT GCC 3'

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE: 3' cDNA primer - C3 sequence

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

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(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

5'GGT GGC GAC CAT CCT CCA AAA 3'

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 888 BASE PAIRS

(B) TYPE: cDNA

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY: C3APL

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3: (Nucleotide sequence of protein C3APL)

GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT AAA GAA TTC GTG ATG GAA TCC CGC AAA CGC GCA AGG CAG ACA TAC ACC CGG TAC CAG ACT CTA GAG CTA GAG AAG GAG TTT CAC TTC AAT CGC TAC TTG ACC CGT CGG CGA AGG ATC GAG ATC GCC CAC GCC CTG TGC CTC ACG GAG CGC CAG ATA AAG ATT TGG TTC CAG AAT CGG CGC ATG AAG TGG AAG AAG GAG AAC TGA

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 295 AMINO ACIDS

(B) TYPE: PROTEIN

(C) STRANDEDNESS SINGLE:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4: (amino acid sequence of C3APL)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSE
KEAIVSYTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPE
NIMLFRGDDPAYLGTEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGY
ISTSLMNVSQFAGRPIITQFKVAKGSKAGYIDPISAFQGQLEMLLPRHST
YHIDDMRLSSDGKQIIITATMMGTAINPKEFVMESRKRARQTYTRYQTLE
LEKEFHFNRYLTRRRRIEIAHALCLTERQIKIWFQNRRMKWKKEN

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 774 BASE PAIRS

(B) TYPE: cDNA

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5: (Nucleotide sequence of C3APS)

GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT
TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA
TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA
AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA

GCT ATC AAT CCT AAA GAA TTC CGC CAG ATC AAG ATT TGG TTC CAG AAT CGT CGC ATG AAG TGG AAG AAG GTC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 257 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:(amino acid sequence of C3APS)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSE
KEAIVSYTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPE
NIMLFRGDDPAYLGTEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGY
ISTSLMNVSQFAGRPIITQFKVAKGSKAGYIDPISAFQGQLEMLLPRHST
YHIDDMRLSSDGKQIIITATMMGTAINPKEFRQIKIWFQNRRMKWKKVDS
SGRIVTD

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 BASE PAIRS

(B) TYPE:

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: 5' cDNA primer - antennapedia sequence

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7: (EX 3)

5'GAA TCC CGC AAA CGC GCA AGG CAG 3'

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 BASE PAIRS

(B) TYPE:NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: 3' cDNA primer

(v) FRAGMENT TYPE: antennapedia sequence

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8: (EX 3)

5'TCA GTT CTC CTT CTT CCA CTT CAT GCG 3'

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 54 BASR PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY:LINEAR

(ii) MOLECULE TYPE: OLIGONEUCLEOTIDE STRAND 1

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9: (EX 3)

5'AAT TCC GCC AGA TCA AGA TTT GGT TCC AGA ATC GTC GCA TGA AGT GGA AGA AGG 3'

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 54 BSE PAIRS

(B) TYPE:NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: OLIGONUCLEOTIDE STRAND 2

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10: (EX 3)

5'GGC GGT CTA GTT CTA AAC CAA GCT CTT AGC AGC GTA GTT CAC CTT CTT CCA GCT 3'

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: TAT 5' PRIMER

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

5'GAATCCAAGCATCCAGGAAGTCAGCC 3'

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:TAT 3' PRIMER

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12: (ex 8)

5' ACC AGC CAC CAC CTT CTG ATA 3'

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 876 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3-TL SEQUENCE

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13: (DNA sequence of C3-TL)

GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA

GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT
TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA
TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA
AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
GCT ATC AAT CCT AAA GAA TTC AAG CAT CCA GGA AGT CAG CCT AAA ACT
GCT TGT ACC AAT TGC TAT TGT AAA AAG TGT TGC TTT CAT TGC CAA GTT
TGT TTC ATA ACA AAA GCC TTA GGC ATC TCC TAT GGC AGG AAG CGG AGA
CAG CGA CGA AGA GCT CAT CAG AAC AGT CAG ACT CAT CAA GCT TCT CTA
TCA AAG CAG TAA

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 291 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3-TL PROTEIN SEQUENCE

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14: (The protein sequence of C3-TL)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
YTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPENIMLFRGDDPAYLG
TEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITQFKVA
KGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFK
HPGSQPKTACTNCYCKKCCFHCQVCFITKALGISYGRKRRQRRRAHQNSQTHQASLS

KQ

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: TS OLIGONUCLEOTIDE STRAND1

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15: (EX 9)

5'AAT TCT ATG GTC GTA AAA AAC GTC GTC AAC GTC GTC GTG 3'

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: TS OLIGONUCLEOTIDE STRAND 2

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16: (EX 9)

5' GAT ACC AGC ATT TTT TGC AGC AGT TGC AGC AGC ACA GCT 3'

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 756 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3-TS cDNA

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17: (Nucleotide sequence of C3-TS)

GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
AAG ACC CCT GAA AAT ATT ATG TTA TTT AGA GGC GAC GAC CCT GCT TAT
TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT AAA GAT
AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTC TCT CAA
TTT GCA GGA AGA CCA ATT ATT ACA CAA TTT AAA GTA GCA AAA GGC TCA
AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
GCT ATC AAT CCT AAA GAA TTC TAT GGT GCT AAA AAA CGT CGT CAA CGT
CGT CGT GTC GAC TCG AGC GGC CCG CAT CGT GAC TGA

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 251 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3-TS PROTEIN

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18: (The protein sequence of C3-TS)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
YTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPENIMLFRGDDPAYLG
TEFQNTLLNSNGTINKTAFEKAKAKFLNKDRLEYGYISTSLMNVSQFAGRPIITQFKVA
KGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFY
GAKKRRQRRRVDSSGPHRD

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:

(B) TYPE:

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19: (EX 10)

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCACTCGAC
TTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGA
AGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTT
CCTTATTATATTGATGGTGATGTTAAATTAACACAGTCTATGGCCATCATACGTTA
TATAGCTGACAAGCACAACATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGAT
TTCAATGCTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTTCGAGAATTGCA
TATAGTAAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAA
TGCTGAAAATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCA
TGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGG
ACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTGA
AGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCATGGCCT
TTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGATC
TGGTTCCGCGTGGATCCTCTAGAGTCGACCTGCAGGCATGCAATGCTTATTCCAT
TAATCAAAAGGCTTATTCAAATACTTACCAGGAGTTTACTAATATTGATCAAGCA
AAAGCTTGGGGTAATGCTCAGTATAAAAAGTATGGACTAAGCAAATCAGAAAAA
GAAGCTATAGTATCATATACTAAAAGCGCTAGTGAAATAAATGGAAAGCTAAGA
CAAAATAAGGGAGTTATCAATGGATTTCCTTCAAATTTAATAAAACAAGTTGAAC
TTTTAGATAAATCTTTTAATAAAATGAAGACCCCTGAAAATATTATGTTATTTAN
AGGCGACGACCCTGCTTATTTAGGAACAGAATTTCAAAACACTCTTCTTAATTCA
AATGGTACAATTAATAAAACGGCTTTTGAAAAGGCTAAAGCTAAGTTTTTAAATA
NAGATAGACTTGAATATGGATATATTAGTACTTCATTAATGAATGTTTCTCAATTT
GCAGGAAGACCAATTATTACAAAATTTAAAGTAGCAAAAGGCTCAAAGGCAGGA
TATATTGACCCTATTAGTGCTTTTCAGGGACAACTTGAAATGTTGCTTCCTAGACA

TAGTACTTATCATATAGACGATATGAGATTGTCTTCTGATGGTAAACAAATAATA
ATTACAGCAACAATGATGGGCACAGCTATCAATCCTAAAGAATTCAGAAGGAAA
CAAAGAAGAAAAAGAAGACTGCAGGCGGCCGCATCGTGA

(2) INFORMATION FOR SEQ ID NO: 20: (EX 10)

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:

(B) TYPE:

(C) STRANDEDNESS:

(D) TOPOLOGY:

(ii) MOLECULE TYPE:

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20: (EX 10)

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPY
YIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDF
ETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTHPDFMLYDALD
VVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPP
KSDLVPRGSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKS
EKEAIVSYTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKM
KTPENIMLFXGDDPAYLGTEFQNTLLNSNGTINKTAFEKAKAKFLNXDRLEYGYISTS
LMNVSQFAGRPIITKFKVAKGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDG
KQIIITATMMGTAINPKEFRRKQRRKRRLQAAAS

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:16 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC1 TRANSPORT SEQUENCE

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21: (C3basic1)

K R R R R R P K K R R R A K R R

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:48 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: OLIGONUCLEOTIDE STRAND 1

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22: (EX 12)

AAG AGA AGG CGA AGA AGA CCT AAG AAG AGA CGA AGG GCG AAG AGG AGA

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 48 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY:LINEAR

(ii) MOLECULE TYPE: C3BASIC1 OLIGONUCLEOTIDE STRAND 2

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23: (EX 12)

TTC TCT TCC GCT TCT TCT GGA TTC TTC TCT GCT TCC CGC TTC TCC TCT

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 792 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC1

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24: (EX 12 DNA sequence)

GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT
CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA
GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA
TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA
AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA
AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG
AAG ACC CCT GAA AAT ATT ATG TTA TTT ANA GGC GAC GAC CCT GCT TAT
TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT
AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT ANA GAT
AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA
TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA
AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA
ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG
TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA
GCT ATC AAT CCT AAA GAA TTC AAG AGA AGG CGA AGA AGA CCT AAG
AAG AGA CGA AGG GCG AAG AGG AGA CAC CAC CAC CAC CAC CAC GTC
GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 263 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC1 PROTEIN SEQUENCE

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25: (EX 12 Protein sequence)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
YTKSASEINGKLRQNKGVINGFPS
NLIKQVELLDKSFNKMKTPENIMLFXGDDPAYLGTEFQNTLLNSNGTINKTAFEKAK
AKFLNXDRLEYGYISTSLMNVSQ
FAGRPIITKFKVAKGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITAT
MMGTAINPKEFKRRRRRPKK
RRRAKRRHHHHHHVDSSGRIVTD.

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 AMINO ACIDS

(B) TYPE:AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC2 TRANSPORT SEQUENCE

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26: (EX 13)

K R R R R K K R R Q R R R

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC2 OLIGONEUCLOTDE STRAND 1

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27: (EX 13)

AAG CGT CGA CGT AGA AAG AAA CGT AGA CAG CGT AGA CGT

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 36 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC 2 OLIGONUCLEOTIDE STRAND 2

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28: (EX 13)

TTC GCA GCT GCA TCT TTC TTT GCA TCT GTC GCA TCT GCA

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 783 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC2 cDNA

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29: (EX 13 DNA sequence)

GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA AAT ATT ATG TTA TTT ANA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT ANA GAT AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA

AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT AAA G AA TTC AAG CGT CGA CGT AGA AAG AAA CGT AGA CAG CGT AGA CGT CAC CAC CAC CAC CAC CAC GTC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 260 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC2 PROTEIN

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30: (EX 13 Protein sequence

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
YTKSASEINGKLRQNKGVINGFPS
NLIKQVELLDKSFNKMKTPENIMLFXGDDPAYLGTEFQNTLLNSNGTINKTAFEKAK
AKFLNXDRLEYGYISTSLMNVSQ
FAGRPIITKFKVAKGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITAT
MMGTAINPKEFKRRRRKKRR
QRRRHHHHHHVDSSGRIVTD.

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC3 TRANSPORT PEPTIDE

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

RRKQRRKRR

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC3 OLIGONUCLEOTIDE STRAND 1

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32: (EX 14)

AGA AGG AAA CAA AGA AGA AAA AGA AGA

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC3 OLIGONUCLEOTIDE STRAND 2

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33: (EX 14)

TCT TCC TTT GTT TCT TCT TTT TCT TCT

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 771 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA C3BASIC3

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:
(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:
(A) NAME/KEY:
(B) LOCATION:
(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:
(A) AUTHORS:
(B) TITLE:
(C) JOURNAL:
(D) VOLUME:
(E) ISSUE:
(F) PAGES:
(G) DATE:
(H) DOCUMENT NO.:
(I) FILING DATE:
(J) PUBLICATION DATE:
(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34: (EX 14 DNA sequence)

GGA TCC TCT AGA GTC GAC CTG CAG GCA TGC AAT GCT TAT TCC ATT AAT CAA AAG GCT TAT TCA AAT ACT TAC CAG GAG TTT ACT AAT ATT GAT CAA GCA AAA GCT TGG GGT AAT GCT CAG TAT AAA AAG TAT GGA CTA AGC AAA TCA GAA AAA GAA GCT ATA GTA TCA TAT ACT AAA AGC GCT AGT GAA ATA AAT GGA AAG CTA AGA CAA AAT AAG GGA GTT ATC AAT GGA TTT CCT TCA AAT TTA ATA AAA CAA GTT GAA CTT TTA GAT AAA TCT TTT AAT AAA ATG AAG ACC CCT GAA AAT ATT ATG TTA TTT ANA GGC GAC GAC CCT GCT TAT TTA GGA ACA GAA TTT CAA AAC ACT CTT CTT AAT TCA AAT GGT ACA ATT AAT AAA ACG GCT TTT GAA AAG GCT AAA GCT AAG TTT TTA AAT ANA GAT AGA CTT GAA TAT GGA TAT ATT AGT ACT TCA TTA ATG AAT GTT TCT CAA TTT GCA GGA AGA CCA ATT ATT ACA AAA TTT AAA GTA GCA AAA GGC TCA AAG GCA GGA TAT ATT GAC CCT ATT AGT GCT TTT CAG GGA CAA CTT GAA ATG TTG CTT CCT AGA CAT AGT ACT TAT CAT ATA GAC GAT ATG AGA TTG TCT TCT GAT GGT AAA CAA ATA ATA ATT ACA GCA ACA ATG ATG GGC ACA GCT ATC AAT CCT AAA GAA TTC AGA AGG AAA CAA AGA AGA AAA AGA AGA CAC CAC CAC CAC CAC CAC G TC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 256 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: C3BASIC3

(v) FRAGMENT TYPE:

(vi) ORIGINAL SOURCE:

(A) ORGANISM:

(vii) IMMEDIATE SOURCE:

(ix) FEATURE:

(A) NAME/KEY:

(B) LOCATION:

(D) OTHER INFORMATION:

(x) PUBLICATION INFORMATION:

(A) AUTHORS:

(B) TITLE:

(C) JOURNAL:

(D) VOLUME:

(E) ISSUE:

(F) PAGES:

(G) DATE:

(H) DOCUMENT NO.:

(I) FILING DATE:

(J) PUBLICATION DATE:

(K) RELEVANT RESIDUES IN SEQ ID NO:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35: (EX 14)

GSSRVDLQACNAYSINQKAYSNTYQEFTNIDQAKAWGNAQYKKYGLSKSEKEAIVS
YTKSASEINGKLRQNKGVINGFPSNLIKQVELLDKSFNKMKTPENIMLFXGDDPAYLG
TEFQNTLLNSNGTINKTAFEKAKAKFLNXDRLEYGYISTSLMNVSQFAGRPIITKFKVA

KGSKAGYIDPISAFQGQLEMLLPRHSTYHIDDMRLSSDGKQIIITATMMGTAINPKEFR
RKQRRKRRHHHHHHVDSSGRIVTD.

5

I claim:

1. A drug delivery construct or conjugate comprising at least one transport agent region and an active agent region not naturally associated with the active agent region, wherein the transport agent region is able to facilitate the uptake of the active agent region into a mammalian tissue or cell, and wherein the active agent region is an active therapeutic agent region able to facilitate axon growth , including a derivative or homologue thereof.

2. A drug delivery construct or conjugate as defined in claim 1 wherein the active agent region is an ADP-ribosyl transferase C3 region.

3. A drug delivery construct or conjugate as defined in claim 2 wherein said ADP-ribosyl transferase C3 is selected from the group consisting of ADP-ribosyl transferase derived from *Closteridum botulinum* and a recombinat ADP-ribosyl transferase.

4. A drug conjugate consisting of a transport polypeptide moiety covalently linked to an active cargo moiety wherein the transport polypeptide moiety is able to facilitate the uptake of the active cargo moiety into a mammalian tissue or cell and wherein the active cargo moiety is an active therapeutic moiety able to facilitate axon growth.

5. A drug conjugate as defined in claim 4 wherein the transport polypeptide moiety is selected from the group consisting of a transport subdomain of HIV Tat protein, the homeodomain of antennopedia, and a functional derivative and analog thereof and wherein the active cargo moiety is selected from the group consisting of C3 protein able to facilitate axon growth.

6. A drug conjugate as defined in claim 5 wherein the C3 protein is ADP-ribosyl transferase C3.

7. A drug conjugate as defined in claim 6 wherein said ADP-ribosyl transferase C3 is selected from the group consisting of ADP-ribosyl transferase derived from

Closteridum botulinum and a recombinat ADP-ribosyl transferase.

8. A drug conjugate as defined in claim 4 wherein the transport polypeptide moiety includes an active contiguous amine acid sequence as described herein

9. A fusion protein consisting of a carboxy terminal active cargo moiety and an amino terminal transport moiety, wherein the terminal transport moiety is selected from the group consisting of a transport subdomain of HIV Tat protein, a transport homeoprotein, and pharmaceutically acceptable functional derivatives and analogs thereof and wherein the active cargo moiety consists of a C3 protein.

10. A fusion protein as defined in claim 9 wherein the C3 protein is ADP-ribosyl transferase C3.

11. A fusion protein as defined in claim 10 wherein said ADP-ribosyl transferase C3 is selected from the group consisting of ADP-ribosyl transferase derived from *Closteridum botulinum* and a recombinat ADP-ribosyl transferase.

12. A fusion protein consisting of a carboxy terminal active cargo moiety and an amino terminal transport moiety, wherein the terminal transport moiety consists of the homeodomain of antennopedia and the active cargo moiety consists of a C3 protein.

13. A fusion protein as defined in claim 12 wherein the C3 protein is ADP-ribosyl transferase C3.

14. A fusion protein as defined in claim 13 wherein said ADP-ribosyl transferase C3 is selected from the group consisting of ADP-ribosyl transferase derived from *Closteridum botulinum* and a recombinat ADP-ribosyl transferase.

15. A fusion protein consisting of a carboxy terminal active cargo moiety and an amino terminal transport moiety, wherein the terminal transport moiety consists of a transport

subdomain of HIV Tat protein and the active cargo moiety consists of a C3 protein.

16. A fusion protein as defined in claim 15 wherein the C3 protein is ADP-ribosyl transferase C3.

17. A fusion protein as defined in claim 16 wherein said ADP-ribosyl transferase C3 is selected from the group consisting of ADP-ribosyl transferase derived from *Closteridum botulinum* and a recombinat ADP-ribosyl transferase.

18. The use of a member selected from the group consisting of a drug delivery construct as defined in any one of claims 1, 2 or 3, a drug conjugate as defined in any one of claims 4, 5, 6, 7 or 8 and a fusion protein as defined in any one of claims 9, to 17 (e.g. including pharmaceutically acceptable chemical equivalents thereof) for suppressing the inhibition of neuronal axon growth.

19. A pharmaceutical composition, the pharmaceutical composition comprising a pharmaceutically acceptable diluent or carrier and an effective amount of a active member selected from the group consisting of a drug delivery construct as defined in any one of claims 1, 2 or 3, a drug conjugate as defined in any one of claims 4, 5, 6, 7 or 8, and a fusion protein as defined in any one of claims 9 to 17 (e.g. including pharmaceutically acceptable chemical equivalents thereof).

20. The use of a member selected from the group consisting of a drug delivery construct as defined in any one of claims 1, 2 or 3, a drug conjugate as defined in any one of claims 4, 5, 6, 7 or 8, and a fusion protein as defined in any one of claims 9 to (e.g. including pharmaceutically acceptable chemical equivalents thereof) for the manufacture of a pharmaceutical composition.

21. A methd for preparing a conjugate or fusion protein as defined above comprising
- cultivating a host cell under conditions hich provide for the expression of the conjugate or fusion protein within the cell ; and
- recovering the conjugate or fusion protein by affinity purification under non-

denaturing conditions.

22. A fusion protein selected from the group consisting of C3APL , C3APS, C3-TL, C3-TS C3-RTS, C3BASIC2 and C3BASIC3 and pharmaceutically acceptable chemical equivalents thereof.

23. A method of suppressing the inhibition of neuronal axon growth comprising delivering a member selected from the group consisting of a drug delivery construct as defined in any one of claims 1, 2 or 3, a drug conjugate as defined in any one of claims 4, 5, 6, 7 or 8 and a fusion protein as defined in any one of claims 9, to 17 to a central nervous system (CNS) lesion site or a peripheral nervous system (PNS) lesion site, in an amount effective to counteract said inhibition.

Figure 1

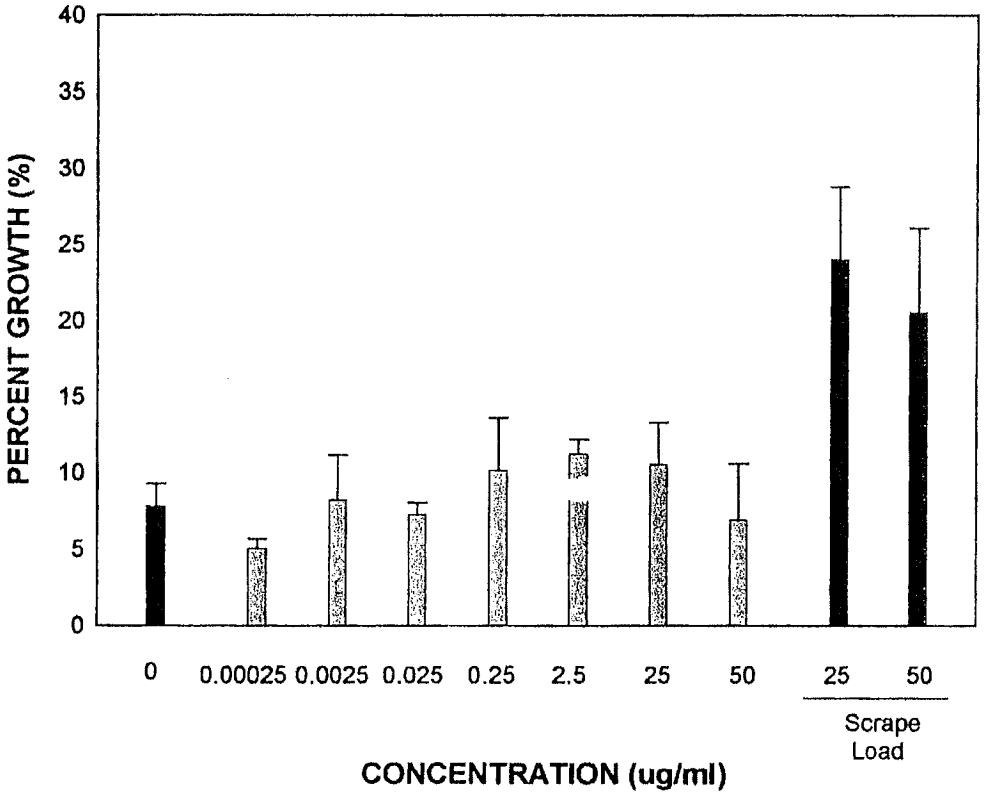

40
35
30
25
20
15
10
5
0
PERCENT GROWTH (%)
0
0.00025
0.0025
0.025
0.25
2.5
25
50
25
50
Scrape
Load
CONCENTRATION (ug/ml)

Figure 2.

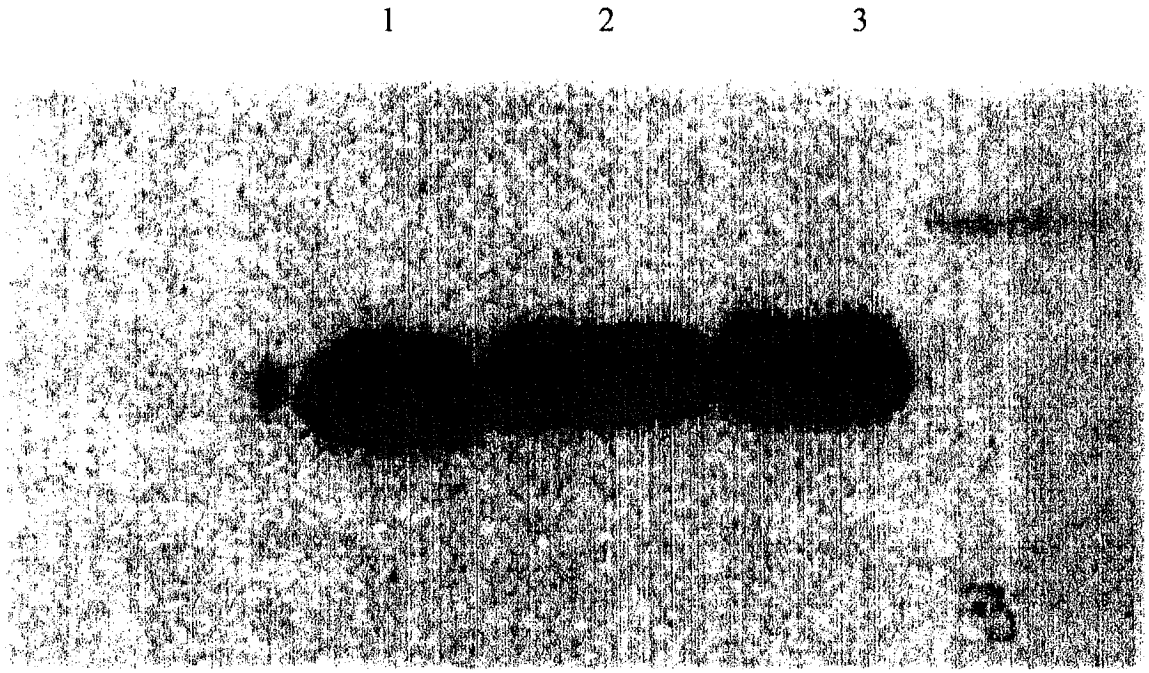


Figure 4

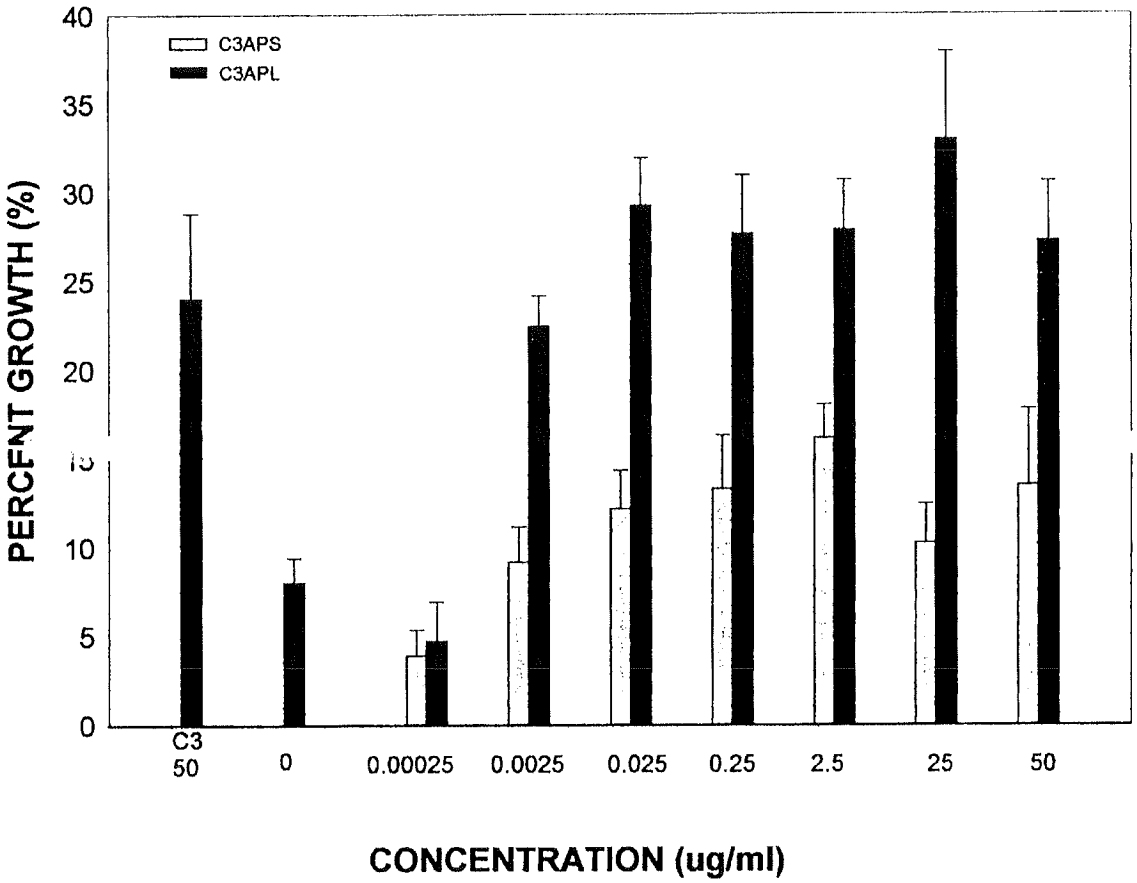
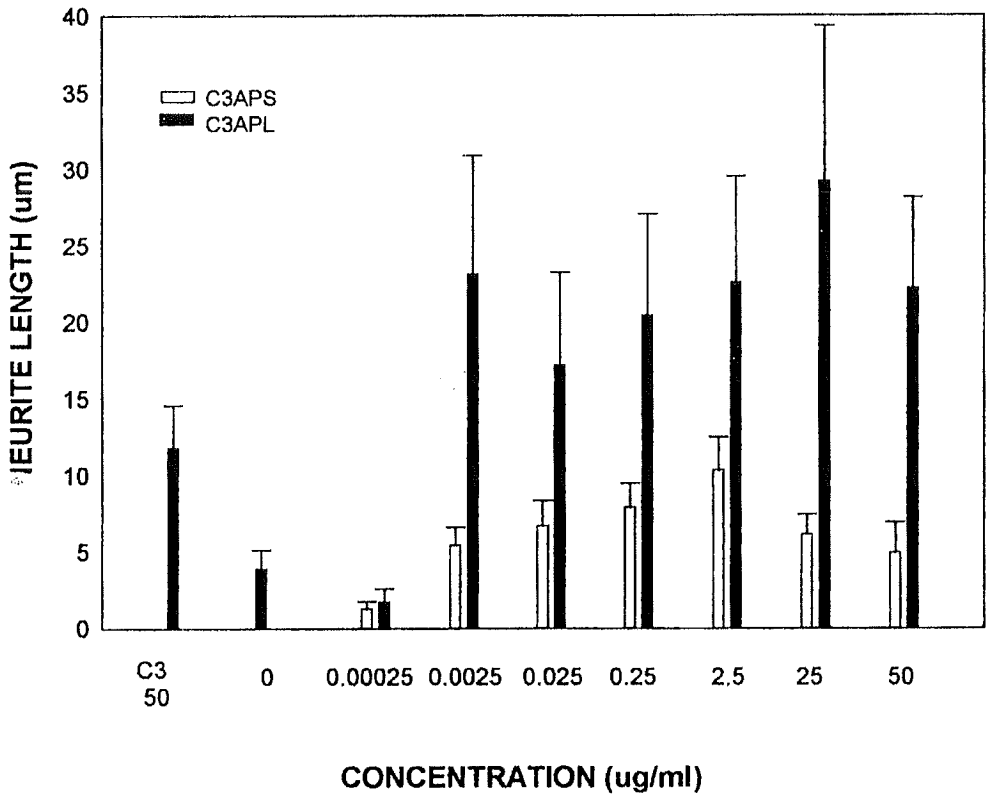

C3APS
C3APL
PERCENT GROWTH (%)
40
35
30
25
20
10
5
0
C3
50
0
0.00025
0.0025
0.025
0.25
2.5
25
50
CONCENTRATION (ug/ml)

Figure 5


NEURITE LENGTH (um)
40
35
30
25
20
15
10
5
0
C3APS
C3APL
C3 50
0
0.00025
0.0025
0.025
0.25
2.5
25
50
CONCENTRATION (ug/ml)

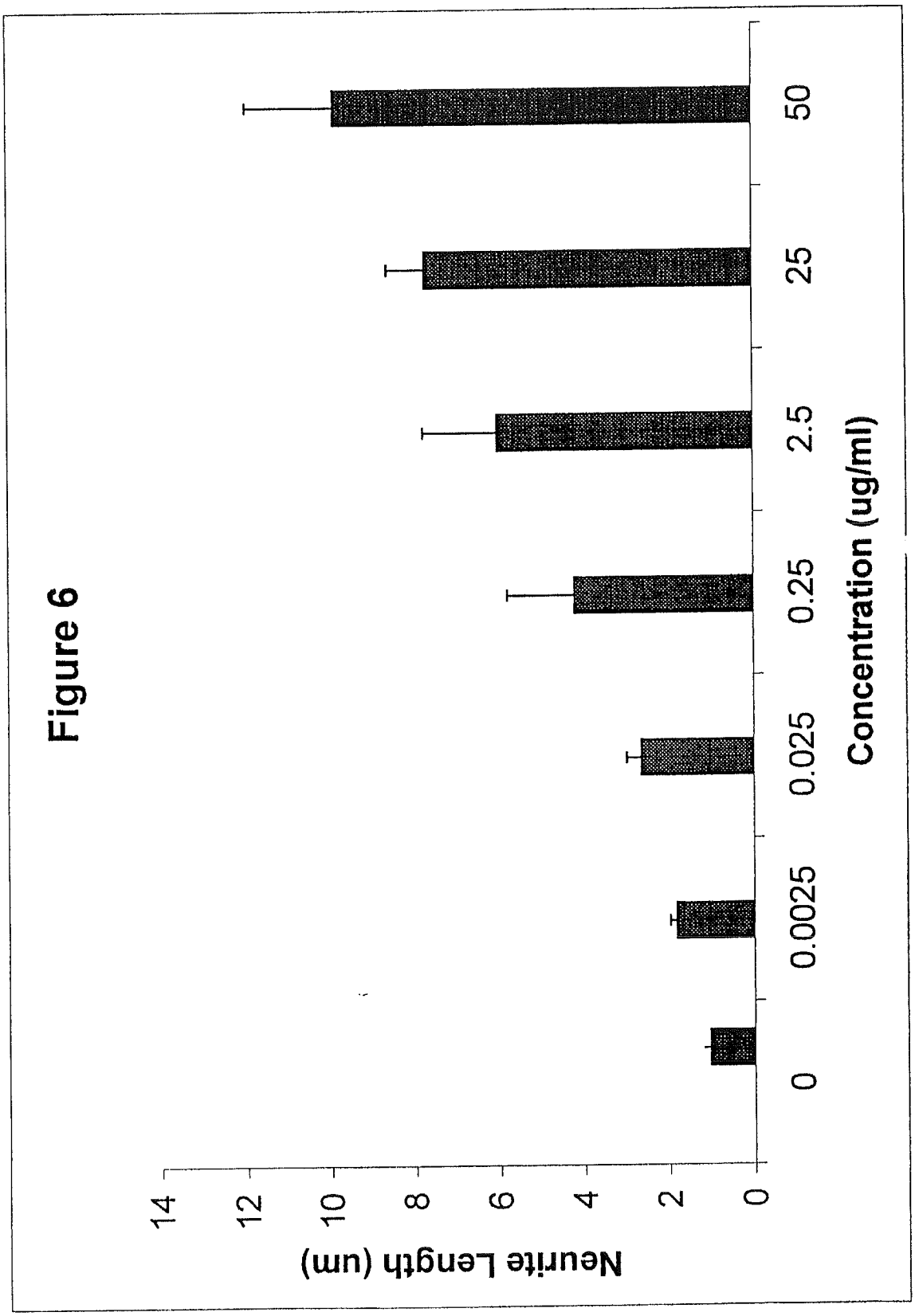


Figure 6
Neurite Length (um)
0
2
4
6
8
10
12
14
0
0.0025
0.025
0.25
2.5
25
50
Concentration (ug/ml)

Figure 7

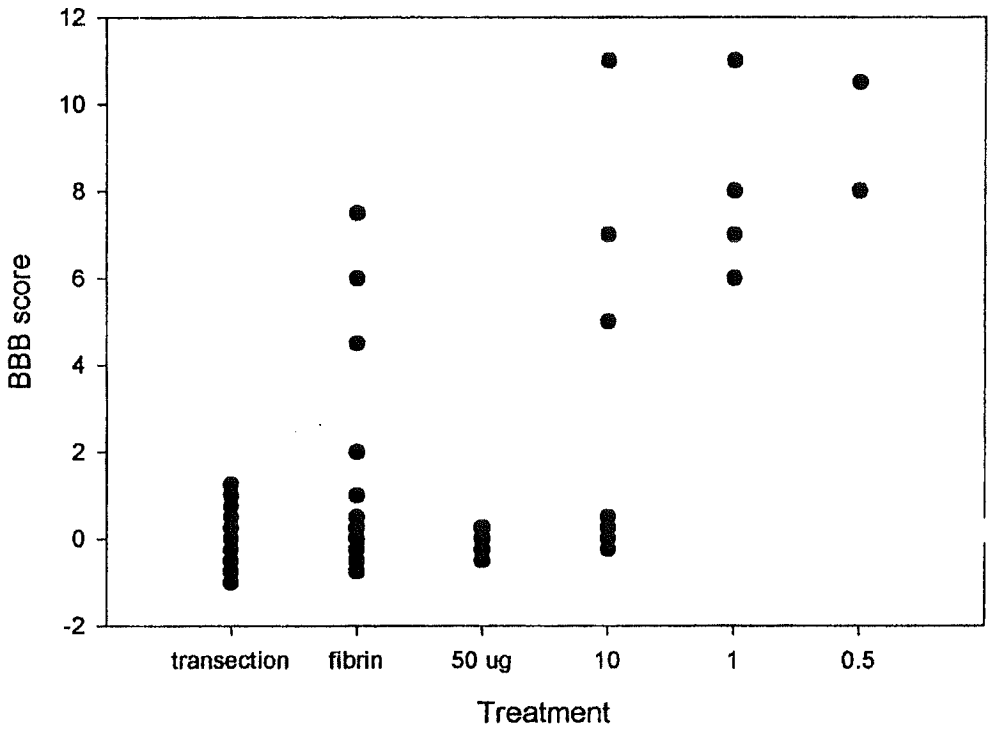
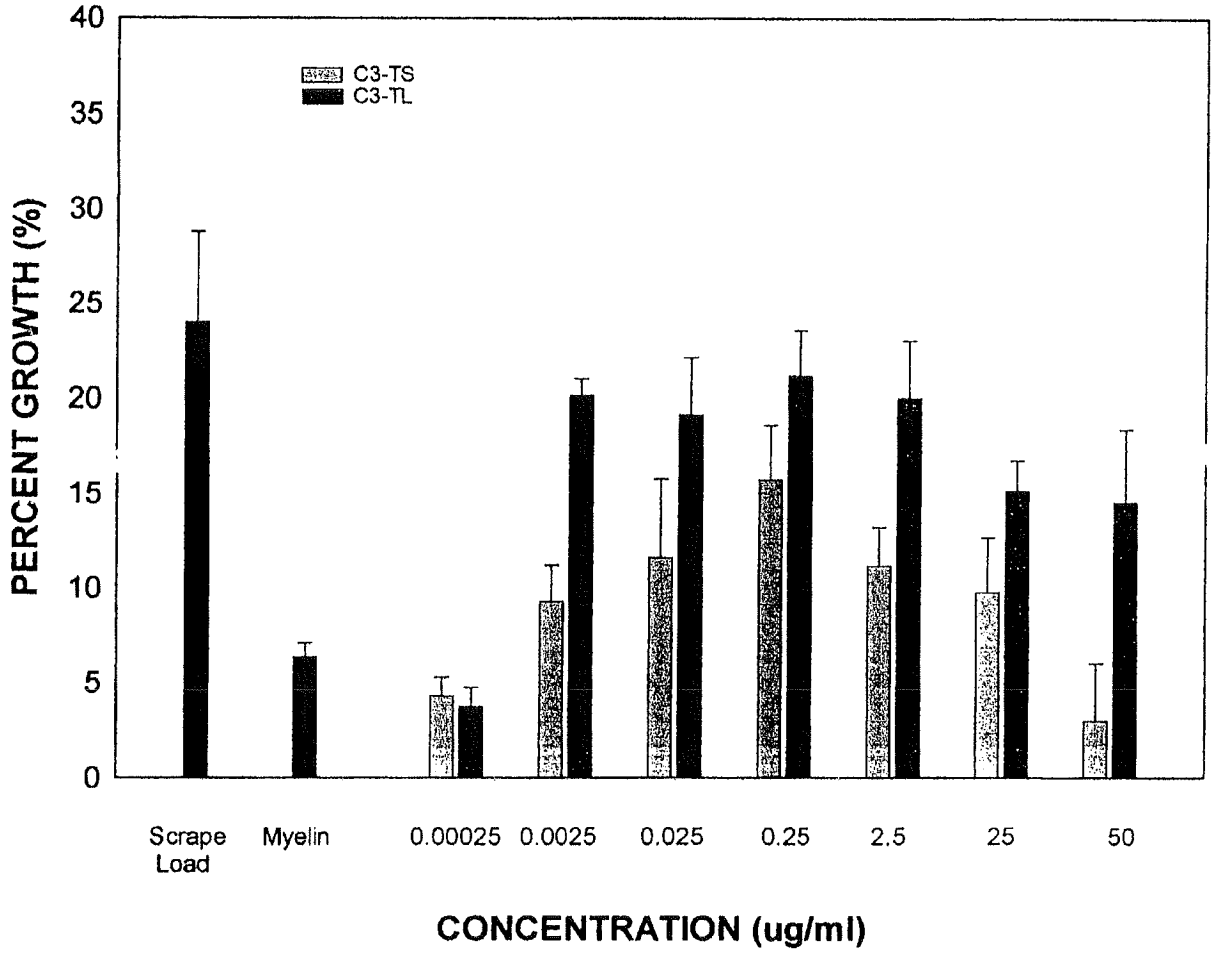

12
10
8
6
4
2
0
-2
BBB score
transection
fibrin
50 ug
10
1
0.5
Treatment

Figure 8


C3-TS
C3-TL
PERCENT GROWTH (%)
0
5
10
15
20
25
30
35
40
Scrape Load
Myelin
0.00025
0.0025
0.025
0.25
2.5
25
50
CONCENTRATION (ug/ml)

UNSCANNABLE ITEM

RECEIVED WITH THIS APPLICATION

(ITEM ON THE 10TH FLOOR ZONE 5 IN THE FILE PREPARATION SECTION)

2342 970

DOCUMENT REÇU AVEC CETTE DEMANDE

NE POUVANT ÊTRE BALAYÉ

(DOCUMENT AU 10 IÈME ÉTAGE AIRE 5 DANS LA SECTION DE LA

PRÉPARATION DES DOSSIERS)